L5 ANSWER 1 OF 74 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002147613 IN-PROCESS
DOCUMENT NUMBER: 21874837 PubMed ID: 11878909
TITLE: Targeted Therapy of Respiratory Syncytial Virus in African Green Monkeys by Intranasally Administered 2-5A Antisense.
AUTHOR: Leaman Douglas W; Longano Frank J; Okicki James R; Soike Kenneth F; Torrence Paul F; Silverman Robert H; Cramer Hagen
CORPORATE SOURCE: Ridgeway Biosystems Inc., 9500 Euclid Avenue, NE50, Cleveland, Ohio, 44195.
SOURCE: VIROLOGY, (2002 Jan 5) 292 (1) 70-7.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20020308
Last Updated on STN: 20020308

AB Respiratory syncytial virus (RSV) is a leading cause of respiratory disease in infants, young children, immunocompromised patients, and the institutionalized elderly. Previous work had shown that **RNase L**, an antiviral enzyme of the interferon system, could be recruited to cleave RSV genomic RNA by attaching tetrameric 2prime prime or minute-5prime prime or minute-linked oligoadenylates (2-5A) to an oligonucleotide complementary to repetitive gene-start sequences within the RSV genome (2-5A **antisense**). A 2prime prime or minute-O-methyl RNA-modified analog of the lead 2-5A anti-RSV chimera is shown here to have enhanced antiviral activity in cell culture studies while also cleaving RSV genomic RNA in an **RNase L**- and sequence-specific manner. When administered intranasally to RSV-infected African green monkeys, this chimera reduced nasal RSV replication by up to four log(10) units in a dose- and time-dependent manner. (C)2002 Elsevier Science.

L5 ANSWER 2 OF 74 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:257990 CAPLUS
DOCUMENT NUMBER: 134:290389
TITLE: **RNase L** activators and **antisense** oligonucleotides effective to treat respiratory syncytial virus infections
INVENTOR(S): Torrence, Paul F.; Silverman, Robert Hugh; Cirino, Nick Mario; Li, Guiying; Xiao, Wei; Player, Mark R.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;
The Cleveland Clinic Foundation
SOURCE: U.S., 63 pp., Cont.-in-part of U.S. 5,998,602.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 6214805 | B1 | 20010410 | US 1997-962690 | 19971103 |
| US 5998602 | A | 19991207 | US 1997-801898 | 19970214 |
| WO 9922742 | A1 | 19990514 | WO 1998-US23391 | 19981102 |

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---|---|---|---|---|
| AU 9913775 | A1 | 19990524 | AU 1999-13775 | 19981102 |
| AU 736470 | B2 | 20010726 | | |
| EP 1033992 | A1 | 20000913 | EP 1998-957541 | 19981102 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2001523636 | T2 | 20011127 | JP 2000-518674 | 19981102 |

PRIORITY APPLN. INFO.:
US 1996-11725P P 19960215
US 1997-801898 A2 19970214
US 1997-962690 A 19971103
WO 1998-US23391 W 19981102

AB Methods are provided for inhibiting infection by RNA viruses with complexes of an activator of **RNase L** and an oligonucleotide that is capable of binding to the genome, antigenome or mRNAs of a neg. strand RNA virus to specifically cleave the genomic or antigenomic RNA strand of the virus. The methods and complexes of the invention may be applied to target any neg. strand RNA virus. In one embodiment, the invention provides a covalently linked complex of an oligonucleotide that is capable of binding to the genomic or antigenomic template RNA strand of a neg. strand RNA virus and/or binding to an mRNA of a viral protein (an "**antisense** oligonucleotide") coupled to an activator of **RNase L**. In a preferred embodiment, the oligonucleotide component of the complex is complementary to a region of the viral genomic RNA strand characterized by repeated or consensus sequences.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L5 ANSWER 3 OF 74 USPATFULL DUPLICATE 3

ACCESSION NUMBER: 2001:126130 USPATFULL
TITLE: Chimeric molecules targeted to viral RNAs
INVENTOR(S): Torrence, Paul F., Flagstaff, AZ, United States
Silverman, Robert H., Beechwood, OH, United States
Maitra, Ratan K., South Euclid, OH, United States
Lesiak, Krystyna, Stone Mountain, GA, United States
PATENT ASSIGNEE(S): The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
The Cleveland Clinic Foundation, Cleveland, OH, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 6271369 | B1 | 20010807 |
| APPLICATION INFO.: | US 1997-950196 | | 19971014 (8) |

RELATED APPLN. INFO.: Division of Ser. No. US 1995-458050, filed on 1 Jun 1995, now patented, Pat. No. US 5677289, issued on 14 Oct 1997 Division of Ser. No. US 1993-123449, filed on 17 Sep 1993, now patented, Pat. No. US 5583032, issued on 10 Dec 1996 Continuation-in-part of Ser. No. US 1992-965666, filed on 21 Oct 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: McGarry, Sean
LEGAL REPRESENTATIVE: Lyon & Lyon LLP, Shalek, Esq., James H., Neuman, Esq., Kristin H.
NUMBER OF CLAIMS: 9

EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 2302
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Chimeric molecules comprising a virus targeting **antisense** oligonucleotide moiety attached to an activator of **2-5A-dependent RNase.**

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 4 OF 74 USPATFULL
ACCESSION NUMBER: 2001:110020 USPATFULL
TITLE: **RNASE L** ACTIVATORS AND **ANTISENSE** OLIGONUCLEOTIDES EFFECTIVE TO TREAT TELOMERASE- EXPRESSING MALIGNANCIES
INVENTOR(S): SILVERMAN, ROBERT H., BEACHWOOD, OH, United States
KONDO, SEIJI, SHAKER HEIGHTS, OH, United States
COWELL, JOHN K., SHAKER HEIGHTS, OH, United States
LI, GUIYING, DURHAM, NC, United States
TORRENCE, PAUL F., SILVER SPRING, MD, United States

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2001007902 | A1 | 20010712 |
| APPLICATION INFO.: | US 1998-18125 | A1 | 19980203 (9) |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 1997-44507P | 19970421 (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: PENNIE & EDMONDS, 1155 AVENUE OF THE AMERICAS, NEW YORK, NY, 10036
NUMBER OF CLAIMS: 29
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 18 Drawing Page(s)
LINE COUNT: 1869
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to chimeric molecules comprising an oligonucleotide complementary to a region of the ribonucleotide component of telomerase attached to an activator of **RNase L** ("activator-**antisense** complex") which specifically cleaves the ribonucleotide portion of a telomerase enzyme. The present invention relates to methods of inhibiting telomerase enzymatic activity with activator-**antisense** complexes targeted to the RNA component of telomerase. The present invention further relates to methods of treating malignant neoplastic disease, wherein the malignant cells contain a telomerase activity that is necessary for the growth of the malignant cells.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 74 USPATFULL
ACCESSION NUMBER: 2001:191118 USPATFULL
TITLE: High affinity DNA binding compounds as adjuvants in antisense technology
INVENTOR(S): Farrell, Nicholas, Richmond, VA, United States
Kloster, Miriam, Richmond, VA, United States
PATENT ASSIGNEE(S): Virginia Commonwealth University, Richmond, VA, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 6310047 | B1 | 20011030 |
| APPLICATION INFO.: | US 1999-379718 | | 19990824 (9) |

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: LeGuyader, John L.
ASSISTANT EXAMINER: Epps, Janet
LEGAL REPRESENTATIVE: McGuireWoods, LLP
NUMBER OF CLAIMS: 20
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 1097
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides an improved method for the delivery and stabilization of antisense oligodeoxynucleotides (ODNs) to cells. The unmodified ODNs are complexed to a polynuclear platinum compound or to a structural derivative thereof. Complexation neutralizes the charge of the ODN and makes possible its passage into the cell, without the addition of other transfection agents. The invention may be used in the treatment any disease which is amenable to treatment by antisense ODNs. In addition, the invention provides a new method specifically for the treatment of cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 6 OF 74 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2001698278 MEDLINE
DOCUMENT NUMBER: 21611192 PubMed ID: 11585831
TITLE: The 2-5A/RNase L/RNase L inhibitor (RNI) pathway regulates mitochondrial mRNAs stability in interferon alpha-treated H9 cells.
AUTHOR: Le Roy F; Bisbal C; Silhol M; Martinand C; Lebleu B; Salehzada T
CORPORATE SOURCE: EP2030 CNRS, Institut de Genetique Moleculaire, 1919 route de Mende, 34293 Montpellier, France.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Dec 21) 276 (51) 48473-82.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200201
ENTRY DATE: Entered STN: 20011218
Last Updated on STN: 20020201
Entered Medline: 20020131

AB Interferon alpha (IFNalpha) belongs to a cytokine family that exhibits antiviral properties, immuno-modulating effects, and antiproliferative activity on normal and neoplasic cells in vitro and in vivo. IFNalpha exerts antitumor action by inducing direct cytotoxicity against tumor cells. This toxicity is at least partly due to induction of apoptosis. Although the molecular basis of the inhibition of cell growth by IFNalpha is only partially understood, there is a direct correlation between the sensitivity of cells to the antiproliferative action of IFNalpha and the down-regulation of their mitochondrial mRNAs. Here, we studied the role of the 2-5A/**RNase L** system and its inhibitor RLI in this regulation of the mitochondrial mRNAs by IFNalpha. We found that a fraction of cellular **RNase L** and RLI is localized in the mitochondria. Thus, we down-regulated **RNase L** activity in human H9 cells by stably transfecting (i) **RNase L antisense** cDNA or (ii) RLI sense cDNA constructions. In contrast to control cells, no post-transcriptional down-regulation of mitochondrial mRNAs and no cell growth inhibition were observed after IFNalpha treatment in these transfectants. These results demonstrate that IFNalpha exerts its antiproliferative effect on H9 cells at least in part via the degradation of mitochondrial mRNAs by **RNase L**.

L5 ANSWER 7 OF 74 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2001426733 MEDLINE
DOCUMENT NUMBER: 21365476 PubMed ID: 11472236
TITLE: Chemistry and biochemistry of 2',5'-oligoadenylate-based antisense strategy.
AUTHOR: Adah S A; Bayly S F; Cramer H; Silverman R H; Torrence P F
CORPORATE SOURCE: Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892, USA.
SOURCE: CURRENT MEDICINAL CHEMISTRY, (2001 Aug) 8 (10) 1189-212. Journal code: C02; 9440157. ISSN: 0929-8673.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011008
Last Updated on STN: 20011008
Entered Medline: 20011004

AB This review describes the application of a natural defense mechanism to develop effective agents for the post-transcriptional control of gene expression. 2-5A is a unique 2',5'-phosphodiester bond linked oligoadenylate, (pp)p5'A2'(p5'A)(n), that is elaborated in virus-infected interferon-treated cells. The 2-5A system is an RNA degradation pathway that is an important mechanistic component of interferon's action against certain viruses. It may also play a role in the anticellular effects of interferon and in general RNA decay. A major player in the 2-5A-system is the latent and constitutive 2-5A-dependent ribonuclease (**RNase L**) which upon activation by 2-5A, degrades RNA. This **RNase L** enzyme can be recruited for **antisense** therapeutics by linking it to an appropriate oligonucleotide targeted to a chosen RNA. Syntheses of 2-5A, its analogues, 2-5A-**antisense**, and its modifications are detailed herein. Applications of 2-5A-**antisense** to particular targets such as HIV, PKR, chronic myelogenous leukemia, telomerase, and respiratory syncytical virus are described.

L5 ANSWER 8 OF 74 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2001231303 MEDLINE
DOCUMENT NUMBER: 21221192 PubMed ID: 11320413
TITLE: Treatment of bladder cancer cells in vitro and in vivo with
2-5A antisense telomerase RNA.
AUTHOR: Koga S; Kondo Y; Komata T; Kondo S
CORPORATE SOURCE: Center for Surgery Research, The Cleveland Clinic Foundation, Cleveland, OH, USA.
SOURCE: GENE THERAPY, (2001 Apr) 8 (8) 654-8. Journal code: CCE; 9421525. ISSN: 0969-7128.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010611
Last Updated on STN: 20010611
Entered Medline: 20010607

AB Bladder cancer is the most common malignant tumor of the urinary tract. Novel treatment approaches are essential because of the failure of current treatment options to cure a high percentage of patients. Telomerase, a ribonucleoprotein, is detected in almost all bladder cancer, but not in normal bladder tissues. Therefore, telomerase is expected to be a very promising candidate for targeted therapy of bladder cancer. In this study, we synthesized a 19-mer **antisense** oligonucleotide against the RNA component of human telomerase (hTR) linked to a 2-5A molecule

(2-5A-anti-hTR) and investigated its antitumor effect against bladder cancer cells. The 2-5A **antisense** strategy relies on the recruitment and activation of **RNase L** at the site of targeted RNA sequence. Here we demonstrate that treatment with 2-5A-anti-hTR reduced the viability of seven bladder cancer cell lines (UM-UC-2, UM-UC-3, UM-UC-6, UM-UC-9, UM-UC-14, RT4 and T24) expressing telomerase activity to 21-55% within 4 days. The cytotoxicity was mainly due to induction of caspase-dependent apoptosis. In contrast, normal fibroblast WI38 cells lacking telomerase activity were resistant to the treatment. Furthermore, treatment of subcutaneous UM-UC-2 tumors in nude mice with 2-5A-anti-hTR significantly suppressed the tumor growth through induction of apoptosis ($P < 0.001$). These findings may offer a strong support to the feasibility of the 2-5A-anti-hTR treatment for human bladder cancer.

L5 ANSWER 9 OF 74 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2001540239 MEDLINE
DOCUMENT NUMBER: 21471541 PubMed ID: 11586893
TITLE: Accelerating RNA decay through intervention of **RNase L**: alternative synthesis of composite 2',5'-oligoadenylate-**antisense**.
AUTHOR: Torrence P F; Wang Z
CORPORATE SOURCE: Department of Chemistry, Northern Arizona University, Flagstaff, Arizona 86011, USA.
SOURCE: METHODS IN ENZYMOLOGY, (2001) 342 20-8.
Journal code: 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 20011008
Last Updated on STN: 20020226
Entered Medline: 20020225

L5 ANSWER 10 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:742300 CAPLUS
DOCUMENT NUMBER: 133:307302
TITLE: Antisense oligonucleotides comprising universal and/or degenerate bases and uses for cleaving target RNA
INVENTOR(S): Brown, Bob D.; Riley, Timothy A.
PATENT ASSIGNEE(S): Oasis Biosciences, Inc., USA
SOURCE: PCT Int. Appl., 32 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000061810 | A1 | 20001019 | WO 2000-US9293 | 20000407 |
| W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| EP 1173614 | A1 | 20020123 | EP 2000-921855 | 20000407 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |

PRIORITY APPLN. INFO.: US 1999-128377P P 19990408
WO 2000-US9293 W 20000407

AB The invention provides **antisense** oligonucleotides contg. one or more degenerate and/or universal bases, and one or more modified backbone linkages, and use of these oligonucleotides for cleaving target RNA mols. The invention also provides **antisense** oligonucleotides designed to recruit RNase including RNase H, **RNase L** or RNase P, where in at least one of the bases in the RNA targeting region of the oligonucleotide are universal and/or degenerate bases. The invention also provides a method for reducing the deleterious effects of an **antisense** oligonucleotide comprising one or more sequence motifs, comprising replacing one or more bases within said one or more sequence motifs with one or more universal and/or degenerate bases.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 11 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:175918 CAPLUS
DOCUMENT NUMBER: 132:232700
TITLE: Peptide nucleic acid-oligoadenylate chimeras, their synthesis and use for inducing RNase L cleavage of RNA
INVENTOR(S): Torrence, Paul F.; Van Boom, Jacques H.; Verheijen, Jeroen C.; Van Der Marel, Gijsbert A.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; Leiden University
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000014219 | A2 | 20000316 | WO 1999-US20159 | 19990902 |
| WO 2000014219 | A3 | 20000706 | | |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9957034 | A1 | 20000327 | AU 1999-57034 | 19990902 |

PRIORITY APPLN. INFO.: US 1998-99173P P 19980904
WO 1999-US20159 W 19990902

AB Covalent conjugation of a 5'-phosphorylated-2',5'-linked oligoadenylate (2-5A) moiety to an **antisense** peptide nucleic acid oligomer (PNA) provides a novel chimeric reagent which effects the selective and specific cleavage of a selected target RNA. The 2-5A-**antisense** PNA chimeras bind the target RNA with high specificity and affinity, and are stable to nucleases. The **antisense** portion of the chimera recruits a chosen RNA as substrate for cleavage, and the 2-5A portion of the chimera binds and activates **RNase L**, thus providing a new approach for the targeted ablation of a target mRNA and a redn. in expression of the protein which it specifies. The chimeric mols. are expected to have utility as research tools and as therapeutic agents. Thus, chimeric mols. comprising p5'A2'p5'A2'p5'A2'p5'A attached to PNA oligoadenylates were synthesized and shown to bind to poly(U) and

stimulate its degrdn. by **RNase L**.

L5 ANSWER 12 OF 74 USPATFULL
ACCESSION NUMBER: 2000:102061 USPATFULL
TITLE: DNA polymerase extension assay
INVENTOR(S): Cole, James L., Doylestown, PA, United States
Kuo, Lawrence C., Solebury, PA, United States
Olsen, David B., Lansdale, PA, United States
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 6100028 | | 20000808 | |
| | WO 9640994 | | 19961219 | |
| APPLICATION INFO.: | US 1998-973139 | | 19980731 | (8) |
| | WO 1996-US8330 | | 19960603 | |
| | | | 19980731 | PCT 371 date |
| | | | 19980731 | PCT 102(e) date |

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Guzo, David
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Yablonsky, Michael D., Tribble, Jack L.
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 5 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 663
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides rapid accurate sensitive assays specific for the detection of at least one a single stranded oligonucleotide produced by the action of an enzyme on a substrate. The assays are useful to detect the presence in a sample of an enzyme which acts on an oligonucleotide substrate to generate a single stranded oligonucleotide product and to detect inhibitors of such an enzyme.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 13 OF 74 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2000:360384 BIOSIS
DOCUMENT NUMBER: PREV200000360384
TITLE: Selective RNA cleavage by isolated **RNase L** activated with 2-5A **antisense** chimeric oligonucleotides.
AUTHOR(S): Silverman, Robert H. (1); Dong, Beihua; Maitra, Ratan K.; Player, Mark R.; Torrence, Paul F.
CORPORATE SOURCE: (1) Department of Cancer, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH, 44195 USA
SOURCE: Phillips, M. Ian. Methods in Enzymology, (2000) Vol. 313, pp. 522-533. Methods in Enzymology; Antisense technology, Part A: General methods, methods of delivery, and RNA studies. print.
Publisher: Academic Press Inc. 525 B Street, Suite 1900, San Diego, CA, 92101-4495, USA.
ISSN: 0076-6879. ISBN: 0-12-182214-1 (cloth).
DOCUMENT TYPE: Book
LANGUAGE: English
SUMMARY LANGUAGE: English

L5 ANSWER 14 OF 74 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2000325320 MEDLINE
DOCUMENT NUMBER: 20325320 PubMed ID: 10866653
TITLE: The 2'-5' oligoadenylate/RNase L/RNase L inhibitor pathway regulates both MyoD mRNA stability and muscle cell differentiation.
AUTHOR: Bisbal C; Silhol M; Laubenthal H; Kaluza T; Carnac G;

Milligan L; Le Roy F; Salehzada T
CORPORATE SOURCE: EP 2030 and UMR 5535 CNRS, 34293 Montpellier Cedex 5, France.. bisbal@jones.igm.cnrs-mop.fr
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (2000 Jul) 20 (14) 4959-69.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000810
Last Updated on STN: 20000810
Entered Medline: 20000724

AB The 2'-5' oligoadenylate (2-5A)/**RNase L** pathway is one of the enzymatic pathways induced by interferon. **RNase L** is a latent endoribonuclease which is activated by 2-5A and inhibited by a specific protein known as RLI (**RNase L** inhibitor). This system has an important role in regulating viral infection. Additionally, variations in **RNase L** activity have been observed during cell growth and differentiation but the significance of the 2-5A/**RNase L**/RLI pathway in these latter processes is not known. To determine the roles of **RNase L** and RLI in muscle differentiation, C2 mouse myoblasts were transfected with sense and **antisense** RLI cDNA constructs. Importantly, the overexpression of RLI in C2 cells was associated with diminished **RNase L** activity, an increased level of MyoD mRNA, and accelerated kinetics of muscle differentiation. Inversely, transfection of the RLI **antisense** construct was associated with increased **RNase L** activity, a diminished level of MyoD mRNA, and delayed differentiation. In agreement with these data, MyoD mRNA levels were also decreased in C2 cells transfected with an inducible **RNase L** construct. The effect of **RNase L** activity on MyoD mRNA levels was relatively specific because expression of several other mRNAs was not altered in C2 transfectants. Therefore, **RNase L** is directly involved in myoblast differentiation, probably through its role in regulating MyoD stability. This is the first identification of a potential mRNA target for **RNase L**.

L5 ANSWER 15 OF 74 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 2000426006 MEDLINE
DOCUMENT NUMBER: 20424194 PubMed ID: 10969793
TITLE: 2-5A antisense telomerase RNA therapy for intracranial malignant gliomas.
AUTHOR: Mukai S; Kondo Y; Koga S; Komata T; Barna B P; Kondo S
CORPORATE SOURCE: Center for Surgery Research, The Cleveland Clinic Foundation, Ohio 44195, USA.
CONTRACT NUMBER: 1R01CA80233 (NCI)
SOURCE: CANCER RESEARCH, (2000 Aug 15) 60 (16) 4461-7.
Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000914

AB Malignant gliomas are the most common intracranial tumors and are considered incurable. Therefore, exploration of novel therapeutic modalities is essential. Telomerase is a ribonucleoprotein enzyme that is detected in the vast majority of malignant gliomas but not in normal brain

tissues. We, therefore, hypothesized that telomerase inhibition could be a very promising approach for the targeted therapy of malignant gliomas. Thus, 2-5A (5'-phosphorylated 2'-5'-linked oligoadenylate)-linked **antisense** against human telomerase RNA component (2-5A-anti-hTER) was investigated for its antitumor effect on an intracranial malignant glioma model. 2-5A is a mediator of one pathway of IFN actions by activating **RNase L**, resulting in RNA degradation. By linking 2-5A to **antisense**, **RNase L** degrades the targeted RNA specifically and effectively. Prior to the experiments using intracranial tumor models in nude mice, we modified the in vitro and in vivo treatment modality of 2-5A-anti-hTER using a cationic liposome to enhance the effect of 2-5A-anti-hTER. Here we demonstrate that 2-5A-anti-hTER complexed with a cationic liposome reduced the viability of five malignant glioma cell lines to 20-43% within 4 days but did not influence the viability of cultured astrocytes lacking telomerase. Furthermore, treatment of intracranial malignant gliomas in nude mice with 2-5A-anti-hTER was therapeutically effective compared with the control ($P < 0.01$). These findings clearly suggest the therapeutic potentiality of 2-5A-anti-hTER as a novel approach for the treatment of intracranial malignant gliomas.

L5 ANSWER 16 OF 74 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 2000282793 MEDLINE
DOCUMENT NUMBER: 20282793 PubMed ID: 10822370
TITLE: Treatment of prostate cancer in vitro and in vivo with 2-5A-anti-telomerase RNA component.
AUTHOR: Kondo Y; Koga S; Komata T; Kondo S
CORPORATE SOURCE: The Center for Surgery Research, The Cleveland Clinic Foundation, OH 44195, USA.
CONTRACT NUMBER: 1R01CA80233 (NCI)
SOURCE: ONCOGENE, (2000 Apr 27) 19 (18) 2205-11.
Journal code: ONC; 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000616
Last Updated on STN: 20000616
Entered Medline: 20000602

AB Prostate cancer is the most common malignancy of elderly men in the United States. Since there is no curative treatment for advanced prostate cancer, exploration of novel modalities of treatment is essential. Telomerase, a ribonucleoprotein, is detected in the vast majority of prostate cancer, but not in normal or benign prostatic hyperplasia tissues. Thus, telomerase is expected to be a very strong candidate for targeted therapy of prostate cancer. In this study, we synthesized a 19-mer **antisense** oligonucleotide against the RNA component of human telomerase (hTR) linked to a 2-5A molecule (2-5A-anti-hTR) and examined its cytotoxic effect on prostate cancer cells. The 2-5A **antisense** strategy relies on the recruitment and activation of **RNase L** at the site of targeted RNA sequence. We here show that treatment with 2-5A-anti-hTR in the presence of a cationic liposome reduced cell viability of tumor cell lines tested to 9-18% within 6 days. In contrast, normal fibroblast cells were resistant to the treatment. Its effect was mainly due to induction of apoptosis by activated caspase family members. Furthermore, treatment of subcutaneous tumors in nude mice with 2-5A-anti-hTR significantly suppressed the tumor growth through induction of apoptosis ($P<0.001$). The treatment with 2-5A-anti-hTR may be

a promising strategy for the treatment modality of prostate cancer with telomerase activity.

L5 ANSWER 17 OF 74 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 2001211736 MEDLINE
DOCUMENT NUMBER: 21040454 PubMed ID: 11200276
TITLE: Synthesis and **RNAse L** binding and activation of a 2-5A-(5')-DNA-(3')-PNA chimera, a novel potential **antisense** molecule.
AUTHOR: Verheijen J C; Chen L; Bayly S F; Torrence P F; van der Marel G A; van Boom J H
CORPORATE SOURCE: Leiden Institute of Chemistry, Gorlaeus Laboratories, The Netherlands.
SOURCE: NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, (2000 Oct-Dec) 19
(10-12) 1821-30.
Journal code: DMF; 100892832. ISSN: 1525-7770.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010425
Last Updated on STN: 20010425
Entered Medline: 20010419
AB Fully automated solid-phase synthesis gave access to a hybrid in which 5'-phosphorylated-2'-5'-linked oligoadenylate (2-5A) is connected to the 5'-terminus of DNA which, in turn, is linked at the 3'-end to PNA [2-5A-(5')-DNA-(3')-PNA chimera]. This novel **antisense** molecule retains full **RNase L** activation potency while suffering only a slight reduction in binding affinity.

L5 ANSWER 18 OF 74 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 12
ACCESSION NUMBER: 2000:269130 CAPLUS
DOCUMENT NUMBER: 133:74259
TITLE: Incorporation of a 4-hydroxy-N-acetylprolinol nucleotide analogue improves the 3'-exonuclease stability of 2'-5'-oligoadenylate-antisense conjugates
AUTHOR(S): Verheijen, Jeroen C.; Van Roon, Anne-Marie M.; Meeuwenoord, Nico J.; Stuivenberg, Hanneke R.; Bayly, Suzanne F.; Chen, Ling; Van der Marel, Gijsbert A.; Torrence, Paul F.; Van Boom, Jacques H.
CORPORATE SOURCE: Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden, 2300 RA, Neth.
SOURCE: Bioorganic & Medicinal Chemistry Letters (2000), 10(8), 801-804
CODEN: BMCLE8; ISSN: 0960-894X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
OTHER SOURCE(S): CASREACT 133:74259
AB Incorporation of a 4-hydroxy-N-acetylprolinol nucleotide analog at the 3'-terminus of DNA or 2-5A-DNA sequences resulted in a significantly enhanced 3'-exonuclease resistance while the affinity for complementary RNA was only slightly decreased. Furthermore, the binding to and activation of human RNase L by thus modified 2-5A-DNA conjugates was not altered as compared to the parent unmodified 2-5A-DNAs.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 19 OF 74 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 2000061752 MEDLINE
DOCUMENT NUMBER: 20061752 PubMed ID: 10595377

TITLE: Selective RNA cleavage by isolated **RNase L** activated with 2-5A **antisense** chimeric oligonucleotides.
AUTHOR: Silverman R H; Dong B; Maitra R K; Player M R; Torrence P F
CORPORATE SOURCE: Department of Cancer, Lerner Research Institute, Cleveland Clinic Foundation, Ohio 44195, USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
CA44059 (NCI)
SOURCE: METHODS IN ENZYMOLOGY, (2000) 313 522-33.
Journal code: MVA; 0212271. ISSN: 0076-6879.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000131
Last Updated on STN: 20000131
Entered Medline: 20000119

L5 ANSWER 20 OF 74 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 2000123926 MEDLINE
DOCUMENT NUMBER: 20123926 PubMed ID: 10637068
TITLE: 2-5A antisense directed against telomerase RNA produces apoptosis in ovarian cancer cells.
AUTHOR: Kushner D M; Paranjape J M; Bandyopadhyay B; Cramer H; Leaman D W; Kennedy A W; Silverman R H; Cowell J K
CORPORATE SOURCE: Department of Gynecology & Obstetrics, The Cleveland Clinic
Foundation, Cleveland, Ohio, 44195, USA.
CONTRACT NUMBER: 1PO1CA62220 (NCI)
SOURCE: GYNECOLOGIC ONCOLOGY, (2000 Feb) 76 (2) 183-92.
Journal code: FXC; 0365304. ISSN: 0090-8258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20000229
Entered Medline: 20000217

AB OBJECTIVE: **RNase L** is converted to an active form upon binding short 2',5'-oligoadenylates (2-5A). To direct **RNase L** to an RNA target, 2-5A is attached to an **antisense** oligonucleotide (2-5A **antisense**). This chimera can be directed against telomerase-an RNA-protein complex that elongates telomeric DNA and is involved in cellular immortalization. Our objective is to investigate the effect of 2-5A **antisense** by targeting telomerase RNA (hTR) in the ovarian cancer cell line, HEY-1B. METHODS: Baseline **RNase L** levels and telomerase activities were measured in both HEY-1B and normal ovarian epithelial cells (NOE). Cells were treated daily with chimeric oligonuclotides (ODN) directed against four different hTR sites, or control ODNs including nonchimeric **antisense**, 2-5A fused to a mismatched sequence, or inactive 2-5A fused to **antisense**. At 48 h, apoptosis was evaluated using the TUNEL assay. After six daily ODN administrations, telomerase activity was redetermined, and at 7 days viability counts were obtained. RESULTS: Both cell lines expressed similar levels of **RNase L**. Hey-1B displayed telomerase activity while NOE did not. After 7 days of transfection, 2-5A **antisense** ODNs caused profound cell death in the HEY-1B cells, but not in the NOE cells. This effect was seen regardless of hTR target site, and ODN controls showed no significant decrease in cell viability in either cell line. HEY1B cells treated with 2-5A **antisense** against hTR showed a decrease in telomerase activity and a profound

induction of programmed cell death. CONCLUSIONS: The results suggest that 2-5A **antisense** directed against telomerase RNA results in apoptotic cell death in ovarian cancer cells, but not normal ovarian epithelial cells. The 2-5A **antisense** strategy may hold a considerable advantage over the conventional **antisense** approach in targeting cancer-causing genes.


L5 ANSWER 21 OF 74 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:79676 BIOSIS
DOCUMENT NUMBER: PREV200100079676
TITLE: 2-5A antisense telomerase RNA therapy for intracranial malignant gliomas.
AUTHOR(S): Kondo, Y. (1); Mukai, S.; Komata, T.; Kondo, S.
CORPORATE SOURCE: (1) Mount Sinai Med Ctr, New York, NY USA
SOURCE: Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-189.7. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience New Orleans, LA, USA November 04-09, 2000 Society for Neuroscience
. ISSN: 0190-5295.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Malignant gliomas are the most common intracranial tumors and are considered incurable. Therefore, exploration of novel therapeutic modalities is essential. Telomerase is a ribonucleoprotein enzyme that is detected in the vast majority of malignant gliomas, but not in normal brain tissues. We, therefore, hypothesized that telomerase inhibition could be a very promising approach for the targeted therapy of malignant gliomas. Thus, 2-5A (5'-phosphorylated 2'-5'-linked oligoadenylate)-linked **antisense** against human telomerase RNA component (2-5A-anti-hTR) was investigated for its antitumor effect on an intracranial malignant glioma model. 2-5A is a mediator of one pathway of interferon actions by activating **RNase L**, resulting in RNA degradation. By linking 2-5A to **antisense**, **RNase L** degrades the targeted RNA specifically and effectively. Prior to the experiments using intracranial tumor models in nude mice, we modified the in vitro and in vivo treatment modality of 2-5A-anti-hTR using a cationic liposome to enhance the effect of 2-5A-anti-hTR. Here we demonstrate that 2-5A-anti-hTR complexed with a cationic liposome reduced the viability of five malignant glioma cell lines to 20 to 43% within 4 days, but did not influence the viability of cultured astrocytes lacking telomerase. Furthermore, treatment of intracranial malignant gliomas in nude mice with 2-5A-anti-hTR was therapeutically effective compared to the control ($P<0.01$). These findings clearly suggest the therapeutic potentiality of 2-5A-anti-hTR as a novel approach for the treatment of intracranial malignant gliomas. NCI (CA80233)

L5 ANSWER 22 OF 74 USPATFULL DUPLICATE 15
ACCESSION NUMBER: 1999:160218 USPATFULL
TITLE: **RNase L** activators and **antisense** oligonucleotides effective to treat RSV infections
INVENTOR(S): Torrence, Paul F., Silver Spring, MD, United States
Silverman, Robert Hugh, Beachwood, OH, United States
Cirino, Nick Mario, Cleveland Heights, OH, United States
Li, Guiying, Durham, NC, United States
Xiao, Wei, North Potomac, MD, United States
PATENT ASSIGNEE(S): The Cleveland Clinic Fouindation and Government, Cleveland, OH, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5998602 | | 19991207 |
| APPLICATION INFO.: | US 1997-801898 | | 19970214 (8) |

| | NUMBER | DATE |
|---|---|---|
| PRIORITY INFORMATION: | US 1996-11725P | 19960215 (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Guzo, David
ASSISTANT EXAMINER: Larson, Thomas G.
LEGAL REPRESENTATIVE: Pennie & Edmonds LLP
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 32 Drawing Figure(s); 30 Drawing Page(s)
LINE COUNT: 2036
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns a compounds and methods for treating infection with Respiratory Syncytial Virus. The compounds comprise an **antisense** portion, which is complementary to a normally single stranded portion of the RSV antigenomic strand (the mRNA strand), a linker and a oligonucleotide activator of **RNase L**, a ubiquitous non-specific RNase. The method comprised forming a complex of an activated **RNase L** and the **antisense** molecule. The application teaches methods of determining which portions of the RSV antigenomic strand are normally single-stranded. The application teaches that an **antisense** oligonucleotide having the sequence of residues 8281-8299 of the RSV genome is particularly useful to practice the invention and provides in vitro results superior to those obtainable with the conventional drug of choice, ribavirin.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 23 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:317183 CAPLUS
DOCUMENT NUMBER: 131:688
TITLE: **RNase L** activators and **antisense** oligonucleotides effective to treat RSV infections
INVENTOR(S): Torrence, Paul F.; Silverman, Robert H.; Cirino, Nick M.; Li, Guiying; Xiao, Wei; Player, Mark R.
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation, USA; National Institutes of Health
SOURCE: PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9922742 | A1 | 19990514 | WO 1998-US23391 | 19981102 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| US 6214805 | B1 | 20010410 | US 1997-962690 | 19971103 |
| AU 9913775 | A1 | 19990524 | AU 1999-13775 | 19981102 |
| AU 736470 | B2 | 20010726 | | |

EP 1033992 A1 20000913 EP 1998-957541 19981102
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
JP 2001523636 T2 20011127 JP 2000-518674 19981102
PRIORITY APPLN. INFO.: US 1997-962690 A 19971103
US 1996-11725P P 19960215
US 1997-801898 A2 19970214
WO 1998-US23391 W 19981102

AB Methods are provided for inhibiting infection by RNA viruses with complexes of an activator of **RNase L** and an oligonucleotide that is capable of binding to the genome, antigenome or mRNAs of a neg. strand RNA virus to specifically cleave the genomic or antigenomic RNA strand of the virus. The methods and complexes of the invention may be applied to target any neg. strand RNA virus. The invention in one embodiment relates to a covalently linked complex of an oligonucleotide that is capable of binding to the genomic or antigenomic template RNA strand of a neg. strand RNA virus and/or binding to an mRNA of a viral protein (an "**antisense** oligonucleotide") coupled to an activator of **RNase L**. In a preferred embodiment, the oligonucleotide component of the complex is complementary to a region of the viral genomic RNA strand characterized by repeated or consensus sequences.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 24 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:249112 CAPLUS
DOCUMENT NUMBER: 130:277638
TITLE: Construction of a combinatorial antisense library
INVENTOR(S): Riley, Timothy A.; Brown, Bob D.; Arnold, Lyle J.
PATENT ASSIGNEE(S): Oasis Biosciences, Inc., USA
SOURCE: PCT Int. Appl., 71 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9918238 | A1 | 19990415 | WO 1998-US20361 | 19980928 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2304798 | AA | 19990415 | CA 1998-2304798 | 19980928 |
| AU 9895118 | A1 | 19990427 | AU 1998-95118 | 19980928 |
| EP 1019539 | A1 | 20000719 | EP 1998-948573 | 19980928 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2001519170 | T2 | 20011023 | JP 2000-515030 | 19980928 |

PRIORITY APPLN. INFO.: US 1997-60673P P 19971002
US 1998-136080 A2 19980818
WO 1998-US20361 W 19980928

AB Combinatorial libraries comprise first oligonucleotide analogs and second oligonucleotide analogs which are coupled together to form antisense mols. capable of binding target polynucleotides and activating an RNase, and ribozymes capable of cleaving polynucleotides. Thus, a preformed library of oligonucleotide analogs is provided, comprising a set of first

oligonucleotide analogs and a set of second oligonucleotide analogs, the analogs having coupling moieties that provide for coupling each first oligonucleotide analog to a second oligonucleotide analog to form an antisense mol. The oligonucleotide analogs are selected to act, when coupled, as a substrate for an endonuclease that recognizes double-stranded RNA or RNA/DNA hybrids when hybridized to a target nucleic acid. The binding domains need to be long enough to insure that the antisense mol. binds to the target polynucleotide, and is able to recruit and/or activate a nuclease. However, the no. of mols. required for a complete library exponentially with length of the sequence represented. By conceptually sepg. the antisense mols. into two or more pieces, a comprehensive antisense library can be prepd. in advance, rather than synthesizing a plurality of candidate antisense mols. as needed. The size of the library needed is reduced by (1) providing the antisense mols. in at least two components, by substituting one or more universal or degenerate bases for some of the natural bases, and (3) by avoiding certain sequences which are predicted to serve as poor antisense mols. by reason of poor binding ability. Chem. syntheses are described for cleaver and/or anchor synthesis-hybridization motifs, and the invention is exemplified by the prepn. of oligonucleotides targeted to protein kinase C.alpha. or human Bcl-2.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 25 OF 74 USPATFULL

ACCESSION NUMBER: 1999:16133 USPATFULL

TITLE: Transgenic plants co-expressing a functional human 2-5A system

INVENTOR(S): Silverman, Robert H., Shaker Heights, OH, United States
Mitra, Amitava, Lincoln, NE, United States

PATENT ASSIGNEE(S): Cleveland Clinic Foundation, Cleveland, OH, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5866787 | | 19990202 |
| APPLICATION INFO.: | US 1995-487797 | | 19950607 (8) |

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1994-198973, filed on 18 Feb 1994, now abandoned which is a continuation-in-part of Ser. No. US 1993-28086, filed on 8 Mar 1993, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: McElwain, Elizabeth F.
LEGAL REPRESENTATIVE: Rothwell, Figg, Ernst & Kurz, PC
NUMBER OF CLAIMS: 16
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 55 Drawing Figure(s); 37 Drawing Page(s)
LINE COUNT: 4181

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel transgenic plants having the ability to express a functional 2-5A system, i.e., a 2-5A synthetase which produces 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) in response to double stranded RNA (dsRNA), and a 2-5A-dependent (RNase L), are disclosed. The novel transgenic plants expressing the functional 2-5A system, such as novel transgenic tobacco plants, are immune to and resistant against viral infection. When the novel transgenic tobacco plants are exposed to three different types of plant viruses, i.e., TMV, TEV and AIMV, such viral exposure leads to necrotic local lesions in such transgenic tobacco

plants instead of typical systemic infections.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 26 OF 74 USPATFULL
ACCESSION NUMBER: 1999:16129 USPATFULL
TITLE: Antiviral transgenic plants, vectors, cells and methods
INVENTOR(S): Silverman, Robert H., Shaker Heights, OH, United States
SenGupta, Dibyendu N., Shaker Heights, OH, United States
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation, Cleveland, OH, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5866781 | | 19990202 |
| APPLICATION INFO.: | US 1995-434998 | | 19950508 (8) |

RELATED APPLN. INFO.: Division of Ser. No. US 1994-198973, filed on 18 Feb 1994 which is a continuation-in-part of Ser. No. US 1993-28086, filed on 8 Mar 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: McElwain, Elizabeth
LEGAL REPRESENTATIVE: Holland & Knight
NUMBER OF CLAIMS: 6
EXEMPLARY CLAIM: 4
NUMBER OF DRAWINGS: 37 Drawing Figure(s); 27 Drawing Page(s)
LINE COUNT: 2853
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated 2-5A-dependent RNases, an interferon-induced enzyme which is activated by 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) and implicated in both the molecular mechanisms of interferon action and in the fundamental control of RNA stability in mammalian cells, and encoding sequences therefor are disclosed. The expression cloning and analysis of murine and human 2-5A-dependent RNases is also disclosed. Recombinant human 2-5A-dependent RNase produced in vitro bound an activating affinity matrix, 2-5A-cellulose, resulting in ribonuclease activity. The 2-5A binding properties of the recombinant and naturally occurring forms of 2-5A-dependent RNase are basically identical. Interferon induction of 2-5A-dependent RNase expression is demonstrated by measuring the mRNA levels in cells treated with interferon and cycloheximide. Analysis of aligned murine and human 2-5A-dependent RNase sequences revealed several features, including similarity to RNase E which is implicated in the control of mRNA stability in E. coli. A duplicated phosphate-binding loop motif is determined by deletion analysis and site-directed mutagenesis to function in the binding of 2-5A. In addition, recombinant nucleotide sequences, recombinant vectors, recombinant cells and antiviral plants which express, for example, amino acid sequences which have activity that interfere with or inhibit viral replication are disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 27 OF 74 USPATFULL
ACCESSION NUMBER: 1999:7287 USPATFULL
TITLE: Antiviral transgenic plants, vectors, cells and methods
INVENTOR(S): Silverman, Robert H., Shaker Heights, OH, United States
SenGupta, Dibyendu N., Shaker Heights, OH, United States
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation, Cleveland, OH, United

States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5861300 | | 19990119 |
| APPLICATION INFO.: | US 1995-436771 | | 19950508 (8) |

RELATED APPLN. INFO.: Division of Ser. No. US 1994-198973, filed on 18 Feb 1994 which is a continuation-in-part of Ser. No. US 1993-28086, filed on 8 Mar 1993, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: McElwain, Elizabeth
LEGAL REPRESENTATIVE: Holland & Knight
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1,5
NUMBER OF DRAWINGS: 37 Drawing Figure(s); 27 Drawing Page(s)
LINE COUNT: 3391
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Isolated 2-5A-dependent RNases, an interferon-induced enzyme which is activated by 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) and implicated in both the molecular mechanisms of interferon action and in the fundamental control of RNA stability in mammalian cells, and encoding sequences therefor are disclosed. The expression cloning and analysis of murine and human 2-5A-dependent RNases is also disclosed. Recombinant human 2-5A-dependent RNase produced in vitro bound an activating affinity matrix, 2-5A-cellulose, resulting in ribonuclease activity. The 2-5A binding properties of the recombinant and naturally occurring forms of 2-5A-dependent RNase are basically identical. Interferon induction of 2-5A-dependent RNase expression is demonstrated by measuring the mRNA levels in cells treated with interferon and cycloheximide. Analysis of aligned murine and human 2-5A-dependent RNase sequences revealed several features, including similarity to RNase E which is implicated in the control of mRNA stability in E. coli. A duplicated phosphate-binding loop motif is determined by deletion analysis and site-directed mutagenesis to function in the binding of 2-5A. In addition, recombinant nucleotide sequences, recombinant vectors, recombinant cells and antiviral plants which express, for example, amino acid sequences which have activity that interfere with or inhibit viral replication are disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 28 OF 74 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1999:481851 BIOSIS
DOCUMENT NUMBER: PREV199900481851
TITLE: Using fluorescence resonance energy transfer (FRET) for measuring 2-5a analogues ability to activate RNase L.
AUTHOR(S): Cramer, Hagen; Geselowitz, Daniel A.; Torrence, Paul F. (1)
CORPORATE SOURCE: (1) Section of Biomedical Chemistry, Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD, 20892 USA
SOURCE: Nucleosides & Nucleotides, (June July, 1999) Vol. 18, No. 6-7, pp. 1523-1525.
ISSN: 0732-8311.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The development of a method for measuring the ability of 2-5A analogues to activate the cleavage of an oligoribonucleotide substrate by RNase L is described. This method is based on fluorescence resonance energy transfer.
The method is easily performed with 96-well plates, allowing for quantitative high-throughput analyses of 2-5A analogues under different

reaction conditions.

L5 ANSWER 29 OF 74 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 1999403437 MEDLINE
DOCUMENT NUMBER: 99403437 PubMed ID: 10474229
TITLE: 2-5A-PNA complexes: a novel class of antisense compounds.
AUTHOR: Verheijen J C; Bayly S F; Player M R; Torrence P F; van der
Marel G A; van Boom J H
CORPORATE SOURCE: Leiden Institute of Chemistry, The Netherlands.
SOURCE: NUCLEOSIDES AND NUCLEOTIDES, (1999 Jun-Jul) 18 (6-7) 1485-6.
Journal code: C5G; 8215930. ISSN: 0732-8311.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19991012
Last Updated on STN: 19991012
Entered Medline: 19990930
AB This paper presents the fully automated solid phase synthesis of 2-5A-PNA hybrids. These stable **antisense** probes cause **RNase L** mediated hydrolysis of target RNA sequences.

L5 ANSWER 30 OF 74 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 1999245669 MEDLINE
DOCUMENT NUMBER: 99245669 PubMed ID: 10230638
TITLE: Discrimination between ribonuclease H- and **ribonuclease L**-mediated RNA degradation by 2'-O-methylated 2-5A-**antisense** oligonucleotides.
AUTHOR: Cramer H; Player M R; Torrence P F
CORPORATE SOURCE: Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and
Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0805, USA.
SOURCE: BIOORGANIC AND MEDICINAL CHEMISTRY LETTERS, (1999 Apr 5) 9 (7) 1049-54.
Journal code: C8B; 9107377. ISSN: 0960-894X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990624
AB 2',5'-Oligoadenylate (2-5A) **antisense** chimeric oligonucleotides were synthesized containing varying 2'-O-methyl-ribonucleotide substitution patterns in the **antisense** domain. The ability of these composite oligonucleotides to mediate RNase H- and **RNase L**-catalyzed RNA degradation showed that these two enzymes have different activation requirements.

L5 ANSWER 31 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:275294 CAPLUS
DOCUMENT NUMBER: 131:53598
TITLE: Phosphorothioate oligodeoxyribonucleotides inhibit ribonuclease L thereby disabling a mechanism of interferon action
AUTHOR(S): Player, Mark R.; Torrence, Paul F.
CORPORATE SOURCE: Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes

of Health, Bethesda, MD, 20892-0805, USA
SOURCE: Bioorg. Med. Chem. Lett. (1999), 9(6), 891-894
CODEN: BMCLE8; ISSN: 0960-894X
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The interferon system is an important early defense against virus infections. Phosphorothioate oligodeoxyribonucleotides were found to be inhibitors of the 2-5A-dependent RNase L. Inhibitory potency depended upon the chain length of the phosphorothioate oligonucleotide and was dependent on the phosphorothioate substitution pattern, but was not substantially base-dependent.
REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L5 ANSWER 32 OF 74 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 1999235437 MEDLINE
DOCUMENT NUMBER: 99235437 PubMed ID: 10220031
TITLE: 2,5-oligoadenylate-peptide nucleic acids (2-5A-PNAs) activate RNase L.
AUTHOR: Verheijen J C; van der Marel G A; van Boom J H; Bayly S F; Player M R; Torrence P F
CORPORATE SOURCE: Leiden Institute of Chemistry, Gorlaeus Laboratories, The Netherlands.
SOURCE: BIOORGANIC AND MEDICINAL CHEMISTRY, (1999 Mar) 7 (3) 449-55.
Journal code: B38; 9413298. ISSN: 0968-0896.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990621
AB To potentiate the 2-5A (2',5'-oligoadenylate)-**antisense** and peptide nucleic acid (PNA) approaches to regulation of gene expression, composite molecules were generated containing both 2-5A and PNA moieties. 2-5A-PNA adducts were synthesized using solid-phase techniques. Highly cross-linked polystyrene beads were functionalized with glycine tethered through a p-hydroxymethylbenzoic acid linker and the PNA domain of the chimeric oligonucleotide analogue was added by sequential elongation of the amino terminus with the monomethoxytrityl protected N-(2-aminoethyl)-N-(adenin-1-ylacetyl)glycinate. Transition to the 2-5A domain was accomplished by coupling of the PNA chain to dimethoxytrityl protected N-(2-hydroxyethyl)-N-(adenin-1-ylacetyl)glycinate. Finally, (2-cyanoethyl)-N,N-diisopropyl-4-O-(4,4-dimethoxytrityl)butylphosphor amidite and the corresponding (2-cyanoethyl)-N,N-diisopropylphosphoramidite of 5-O-(4,4'-dimethoxytrityl)-3-O-(tert-butyldimethylsilyl)-N6-benzoyladeno sine were the synthons employed to add the 2 butanediol phosphate linkers and the four 2',5'-linked riboadenylates. The 5'-phosphate moiety was introduced with 2-[[2-(4,4'-dimethoxytrityloxy)ethyl]sulfonyl]ethyl-(2-cyanoethyl) -N,N-diisopropylphosphoramidite. Deprotection with methanolic NH3 and tetraethylammonium fluoride afforded the desired products, 2-SA-pnaA4, 2-5A-pnaA8 and 2-5A-pnaA12. When evaluated for their ability to cause the degradation of two different RNA substrates by the **2-5A -dependent RNase L**, these new 2-5A-PNA conjugates were found to be potent **RNase L** activators. The union of 2-5A and PNA presents fresh opportunities to explore the biological and therapeutic implications of these unique approaches to **antisense**.

L5 ANSWER 33 OF 74 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 19
ACCESSION NUMBER: 1999:548324 CAPLUS
DOCUMENT NUMBER: 131:280845
TITLE: 2',5'-oligoadenylate-antisense chimeras as experimental therapeutic agents for cancer and viral infections
AUTHOR(S): Silverman, Robert H.; Cowell, John K.; Torrence, Paul F.
CORPORATE SOURCE: Department of Cancer Biology, Lerner Research Institute, The Cleveland Clinic Foundation, Cleveland, OH, 44195, USA
SOURCE: Antisense Nucleic Acid Drug Dev. (1999), 9(4), 409-414
CODEN: ANADF5; ISSN: 1087-2906
PUBLISHER: Mary Ann Liebert, Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 23 refs. of recent progress in a strategy that harnesses RNase L (2-5A-dependent RNase) for the purpose of selectively degrading RNA mols. of choice in vivo.
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 34 OF 74 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 1999102585 MEDLINE
DOCUMENT NUMBER: 99102585 PubMed ID: 9847332
TITLE: RNase L inhibitor is induced during human immunodeficiency virus type 1 infection and down regulates the 2-5A/RNase L pathway in human T cells.
AUTHOR: Martinand C; Montavon C; Salehzada T; Silhol M; Lebleu B; Bisbal C
CORPORATE SOURCE: Institut de Genetique Moleculaire de Montpellier (UMR 5535, CNRS-Universite de Montpellier II), 34293 Montpellier Cedex 5, France.
SOURCE: JOURNAL OF VIROLOGY, (1999 Jan) 73 (1) 290-6.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 19990209
Entered Medline: 19990128
AB The interferon-regulated 2-5A/**RNase L** pathway plays a major role in the antiviral and antiproliferative activities of these cytokines. Several viruses, however, have evolved strategies to escape the antiviral activity of the 2-5A/**RNase L** pathway. In this context, we have cloned a cDNA coding for the **RNase L** inhibitor (RLI), a protein that specifically inhibits **RNase L** and whose regulated expression in picornavirus-infected cells down regulates the activity of the 2-5A/**RNase L** pathway. We show here that RLI increases during the course of human immunodeficiency virus type 1 (HIV-1) infection, which may be related to the downregulation of **RNase L** activity that has been described to occur in HIV-infected cells. In order to establish a possible causal relationship between these observations, we have stably transfected H9 cells with RLI sense or **antisense**

cDNA-expressing vectors. The overexpression of RLI causes a decrease in **RNase L** activity and a twofold enhancement of HIV production. This increase in HIV replication correlates with an increase in HIV RNA and proteins. In contrast, reduction of RLI levels in RLI **antisense** cDNA-expressing clones reverses the inhibition of **RNase L** activity associated with HIV multiplication and leads to a threefold decrease in the viral load. This anti-HIV activity correlated with a decrease in HIV RNA and proteins. These findings demonstrate that the level of RLI, via its modulation of **RNase L** activity, can severely impair HIV replication and suggest the involvement of RLI in the inhibition of the 2-5A/**RNase L** system observed during HIV infection.

L5 ANSWER 35 OF 74 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 1999388448 MEDLINE
DOCUMENT NUMBER: 99388448 PubMed ID: 10454983
TITLE: Controlling gene expression with 2-5A antisense.
AUTHOR: Leaman D W; Cramer H
CORPORATE SOURCE: Gemini Technologies Inc., 11,000 Cedar Avenue, Suite 140, Cleveland, Ohio 44106, USA.. dougl@geminitech.com
SOURCE: METHODS, (1999 Jul) 18 (3) 252-65. Ref: 48
Journal code: CPO; 9426302. ISSN: 1046-2023.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990916

AB Recent work has demonstrated that the activity of a ubiquitous cellular enzyme, **ribonuclease L** (**RNase L**), can be harnessed to cleave targeted RNA species. Activation of **RNase L** is dependent on the presence of 2',5'-linked oligoadenylates (2-5A), usually produced by cells infected with viruses. By conjugating synthetic 2-5A to specific **antisense** compounds, it is now possible to selectively degrade RNAs in an **RNase L**-dependent manner, thereby providing an alternative to RNase H-dependent approaches. In this summary, we provide an updated description of the synthesis procedure for constructing these chimeric 2-5A **antisense** molecules. Examples of successful applications of the 2-5A **antisense** strategy are described, along with some of the procedures involved in those studies. Several methods are also provided for optimizing compound uptake and analyzing their effects on cells. Finally, we discuss the current body of evidence that supports the contention that **RNase L** is indeed the primary mediator of 2-5A **antisense** effects and the possible implications that this has on the future of this therapeutic approach.


L5 ANSWER 36 OF 74 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 1999376564 MEDLINE
DOCUMENT NUMBER: 99376564 PubMed ID: 10446388
TITLE: Evidence for IRF-1-dependent gene expression deficiency in interferon unresponsive HepG2 cells.
AUTHOR: Tnani M; Bayard B A
CORPORATE SOURCE: UMR 5539 Centre National de la Recherche Scientifique, Universite de Montpellier II, Place E. Bataillon, Case 107, 34095, Montpellier Cedex 5, France.
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Aug 12) 1451 (1) 59-72.
Journal code: A0W; 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991014
Last Updated on STN: 19991014
Entered Medline: 19991001

AB Induction of the antiproliferative and antiviral state by IFNs (type I and II) is dramatically impaired in HepG2 cells. We show here that **RNase L**, IDO, GBP-2 and iNOS genes normally expressed as a secondary response to IFN are no longer inducible in HepG2 cells, while induction of primary response genes (IRF-1, PKR, p48-ISGF3gamma, 2-5AS, 6-16 and p56-(trp)tRNA) are unaffected. On the basis of previous data implicating transcription factor IRF-1 in the induction of some IFN-induced genes, we tested the effects of transfecting an IRF-1 oligonucleotide **antisense** in HeLa cells and found specifically impaired IFN induction of secondary response genes (**RNase L**, IDO and GBP-2). This raised the possibility that IRF-1 was defective in HepG2 cells. However, some molecular and biochemical analyses reveal that IRF-1 is induced normally by IFNs and retains its normal size, cellular location, phosphorylation status and ability to bind the IDO promoter in vitro. Therefore, we conclude that although the primary response pathway is fully functional, some aspects of the secondary pathway involving IRF-1 (but not IRF-1 itself) are defective in HepG2 cells. It may be possible that the promoter region of these deficient HepG2-genes requires an unidentified transcription factor in addition to de novo IRF-1, which could be elicited by a cooperative activator.

L5 ANSWER 37 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:709089 CAPLUS
DOCUMENT NUMBER: 129:326087
TITLE: **RNase L** activators linked to **antisense** oligonucleotides for effective treatment of telomerase-expressing malignancies
INVENTOR(S): Silverman, Robert H.; Kondo, Seiji; Cowell, John K.; Li, Guiying; Torrence, Paul F.
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation, USA; National Institutes of Health
SOURCE: PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9847911 | A1 | 19981029 | WO 1998-US7397 | 19980413 |
| W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, GW, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| US 2001007902 | A1 | 20010712 | US 1998-18125 | 19980203 |
| AU 9871135 | A1 | 19981113 | AU 1998-71135 | 19980413 |
| EP 975649 | A1 | 20000202 | EP 1998-918160 | 19980413 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 2001524100 | T2 | 20011127 | JP 1998-546125 | 19980413 |

PRIORITY APPLN. INFO.: US 1997-44507P P 19970421

US 1998-18125 A 19980203
WO 1998-US7397 W 19980413

AB The present invention relates to chimeric mols. comprising an oligonucleotide complementary to a region of the ribonucleotide component of telomerase attached to an activator of **RNase L** ("activator-**antisense** complex") which specifically cleaves the ribonucleotide portion of a telomerase enzyme. The activator moiety comprises a 2'-5'-linked oligoadenylate. The present invention relates to methods of inhibiting telomerase enzymic activity with activator-**antisense** complexes targeted to the RNA component of telomerase. The present invention further relates to methods of treating malignant neoplastic disease, wherein the malignant cells contain a telomerase activity that is necessary for the growth of the malignant cells. Thus, Sp5'A(2'p5'A)3-Bu2-5'-GCGCGGGGAGCAAAAGCAC3'-3'T5' (where Bu2 is a bis-1,4-butanediol phosphodiester linker) is effective in the treatment of a variety of tumors, particularly in combination with a chemotherapeutic agent such as cisplatin.

L5 ANSWER 38 OF 74 USPATFULL

| | |
|---|---|
| ACCESSION NUMBER: | 1998:122542 USPATFULL |
| TITLE: | C-myb ribozymes having 2'-5'-linked adenylate residues |
| INVENTOR(S): | Stinchcomb, Dan T., 7203 Old Post Rd., Boulder, CO, United States 80301<br>Draper, Kenneth, 4619 Cloud Ct., Boulder, CO, United States 80301<br>McSwiggen, James, 4866 Franklin Dr., Boulder, CO, United States 80301<br>Jarvis, Thale, 3720 Smuggler Pl., Boulder, CO, United States 80301 |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5817796 | | 19981006 |
| APPLICATION INFO.: | US 1995-435628 | | 19950505 (8) |

RELATED APPLN. INFO.: Division of Ser. No. US 1995-373124, filed on 13 Jan 1995, now patented, Pat. No. US 5646042 And a continuation-in-part of Ser. No. US 1992-987132, filed on 7 Dec 1992, now abandoned Ser. No. Ser. No. US 1994-245466, filed on 18 May 1994, now abandoned And Ser. No. US 1994-192943, filed on 7 Feb 1994 which is a continuation of Ser. No. US 1992-936422, filed on 26 Aug 1992, now abandoned

| | |
|---|---|
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | Granted |
| PRIMARY EXAMINER: | LeGuyader, John L. |
| LEGAL REPRESENTATIVE: | Lyon & Lyon LLP |
| NUMBER OF CLAIMS: | 3 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 21 Drawing Figure(s); 24 Drawing Page(s) |
| LINE COUNT: | 16761 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Enzymatic nucleic acid molecules which cleave c-myb RNA or other RNAs associated with restenosis or cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 39 OF 74 MEDLINE DUPLICATE 23

| | |
|---|---|
| ACCESSION NUMBER: | 1998338009 MEDLINE |
| DOCUMENT NUMBER: | 98338009 PubMed ID: 9671772 |
| TITLE: | Potent inhibition of respiratory syncytial virus replication using a 2-5A-antisense chimera targeted to signals within the virus genomic RNA. |
| AUTHOR: | Player M R; Barnard D L; Torrence P F |

CORPORATE SOURCE: Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0805, USA.
CONTRACT NUMBER: N01-AI35178 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Jul 21) 95 (15) 8874-9. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980820

AB The 2-5A system is a recognized mechanistic component of the antiviral action of interferon. Interferon-induced 2-5A synthetase generates 2-5A, which, in turn, activates the latent constitutive **RNase L** that degrades viral RNA. Chemical conjugation of 2-5A to an **antisense** oligonucleotide can target the **2-5A-dependent RNase L** to the **antisense** -specified RNA and effect its selective destruction. Such a 2-5A-**antisense** chimera (NIH351) has been developed that targets a consensus sequence within the respiratory syncytial virus (RSV) genomic RNA. NIH351 was 50- to 90-fold more potent against RSV strain A2 than was ribavirin, the presently approved drug for clinical management of RSV infection. It was similarly active against a variety of RSV strains of both A and B subgroups and possessed a cell culture selectivity index comparable to ribavirin. In addition, the anti-RSV activity of NIH351 was shown to be virus-specific and a result of a true **antisense** effect, because a scrambled nucleotide sequence in the **antisense** domain of NIH351 caused a significant decrease in antiviral activity. The 2-5A system's **RNase L** was implicated in the mechanism of action of NIH351 because a congener with a disabled 2-5A moiety was of greatly reduced anti-RSV effectiveness. These findings represent an innovative approach to the control of RSV replication.

L5 ANSWER 40 OF 74 MEDLINE DUPLICATE 24
ACCESSION NUMBER: 1999055554 MEDLINE
DOCUMENT NUMBER: 99055554 PubMed ID: 9834240
TITLE: 2',5'-Oligoadenylate-**antisense** chimeras cause **RNase L** to selectively degrade bcr/abl mRNA in chronic myelogenous leukemia cells.
AUTHOR: Maran A; Waller C F; Paranjape J M; Li G; Xiao W; Zhang K; Kalaycio M E; Maitra R K; Lichtin A E; Brugger W; Torrence P F; Silverman R H
CORPORATE SOURCE: Department of Cancer Biology, The Lerner Research Institute, and Department of Hematology and Oncology, Cleveland Clinic Foundation, Cleveland, OH,USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
SOURCE: BLOOD, (1998 Dec 1) 92 (11) 4336-43. Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990115
Last Updated on STN: 19990115
Entered Medline: 19990105

AB We report an RNA targeting strategy, which selectively degrades bcr/abl mRNA in chronic myelogenous leukemia (CML) cells. A 2', 5'-tetraadenylate activator (2-5A) of **RNase L** was chemically linked to oligonucleotide **antisense** directed against either the fusion

site or against the translation start sequence in bcr/abl mRNA. Selective degradation of the targeted RNA sequences was demonstrated in assays with purified **RNase L** and decreases of p210(bcr/abl) kinase activity levels were obtained in the CML cell line, K562. Furthermore, the 2-5A-**antisense** chimeras suppressed growth of K562, while having substantially reduced effects on the promyelocytic leukemia cell line, HL60. Findings were extended to primary CML cells isolated from bone marrow of patients. The 2-5A-**antisense** treatments both suppressed proliferation of the leukemia cells and selectively depleted levels of bcr/abl mRNA without affecting levels of beta-actin mRNA, determined by reverse transcriptase-polymerase chain reaction (RT-PCR). The specificity of this approach was further shown with control oligonucleotides, such as chimeras containing an inactive dimeric form of 2-5A, **antisense** lacking 2-5A, or chimeras with altered sequences including several mismatched nucleotides. The control oligonucleotides had either reduced or no effect on CML cell growth and bcr/abl mRNA levels. These findings show that CML cell growth can be selectively suppressed by targeting bcr/abl mRNA with 2-5A-**antisense** for decay by **RNase L** and suggest that these compounds should be further explored for their potential as ex vivo purging agents of autologous hematopoietic stem cell transplants from CML patients.

L5 ANSWER 41 OF 74 MEDLINE DUPLICATE 25
ACCESSION NUMBER: 1998345157 MEDLINE
DOCUMENT NUMBER: 98345157 PubMed ID: 9681832
TITLE: Targeted therapy of human malignant glioma in a mouse model by 2-5A antisense directed against telomerase RNA.
AUTHOR: Kondo S; Kondo Y; Li G; Silverman R H; Cowell J K
CORPORATE SOURCE: Department of Neurosurgery, Brain Tumor Center/Cancer Center, The Cleveland Clinic Foundation, Ohio 44195, USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
SOURCE: ONCOGENE, (1998 Jun 25) 16 (25) 3323-30.
Journal code: ONC; 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980817
Last Updated on STN: 19980817
Entered Medline: 19980804

AB Telomerase is the RNA-protein complex which elongates telomeric DNA (TTAGGG)n and appears to play an important role in cellular immortalization. The almost exclusive expression of telomerase in tumor cells, and not in most normal cells, offers an exciting opportunity for therapy by inhibiting its function. Here, we have investigated the effect of inhibition of telomerase on the growth and survival of human malignant glioma cells in vitro and in vivo by using a 19-mer **antisense** oligonucleotide against human telomerase RNA linked to a 2',5'-oligoadenylate (2-5A). 2-5A **antisense** functions by activating the endoribonuclease, **RNase L**, resulting in the degradation of single stranded, targeted RNA. We have shown that the 2-5A **antisense** treatment effectively suppressed tumor cell growth and survival in vitro. Furthermore, treatment of tumors grown in nude mice with the **antisense** oligonucleotide inhibited survival of the tumor cells. TUNEL assays suggest that this effect is mediated through the induction of apoptosis. Targeting telomerase RNA with 2-5A **antisense**, therefore, may represent an effective and novel approach for treatment of a broad range of cancers.

L5 ANSWER 42 OF 74 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 1998223642 MEDLINE
DOCUMENT NUMBER: 98223642 PubMed ID: 9554886

TITLE: Nuclease-resistant composite 2',5'-oligoadenylate-3', 5'-oligonucleotides for the targeted destruction of RNA: 2-5A-iso-antisense.
AUTHOR: Xiao W; Li G; Player M R; Maitra R K; Waller C F; Silverman R H; Torrence P F
CORPORATE SOURCE: Section on Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
SOURCE: JOURNAL OF MEDICINAL CHEMISTRY, (1998 Apr 23) 41 (9) 1531-9.
Journal code: J0F; 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980529
Last Updated on STN: 19980529
Entered Medline: 19980521

AB A new modification of 2-5A-**antisense**, 2-5A-iso-**antisense**, has been developed based on a reversal of the direction of the polarity of the **antisense** domain of a 2-5A-**antisense** composite nucleic acid. This modification was able to anneal with its target RNA as well as the parental 2-5A-**antisense** chimera. The 2-5A-iso-**antisense** oligonucleotide displayed enhanced resistance to degradation by 3'-exonuclease enzyme activity such as that represented by snake venom phosphodiesterase and by that found in human serum. 2-5A-Iso-**antisense** was able to effect the degradation of a synthetic nontargeted substrate, [5'-32P]pC11U2C7, and two targeted RNAs, PKR and BCR mRNAs, in a cell-free system containing purified recombinant human **2-5A-dependent RNase L**. These results demonstrated that the novel structural modification represented by 2-5A-iso-**antisense** provided a stabilized biologically active formulation of the 2-5A-**antisense** strategy.

L5 ANSWER 43 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:225111 CAPLUS
DOCUMENT NUMBER: 129:13929
TITLE: RNase L dimerization in a mammalian two-hybrid system in response to 2',5'-oligoadenylates
AUTHOR(S): Naik, Sharon; Paranjape, Jayashree M.; Silverman, Robert H.
CORPORATE SOURCE: Department of Cancer Biology, The Lerner Research Institute, NN1-06, Cleveland Clinic Foundation, Cleveland, OH, 44195, USA
SOURCE: Nucleic Acids Res. (1998), 26(6), 1522-1527
CODEN: NARHAD; ISSN: 0305-1048
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB RNase L, a key enzyme in the anti-viral activity of interferons, requires activation by 2',5'-linked oligoadenylates (2-5A) to cleave viral and cellular single-stranded RNA. Here we demonstrate that 2-5A causes formation of stable dimers of RNase L in intact human cells as measured with a mammalian two-hybrid system. Hybrid proteins consisting of the GAL4 DNA binding domain fused to RNase L and the VP16 transactivation domain fused to RNase L were able to assoc. and drive transcription of a reporter gene, but only after cells were transfected with 2-5A. Several functional forms of 2-5A, such as p3A2'p5'A2'p5'A, were capable of activating transcription in human HeLa cells. In contrast, p3A2'p5'A, which can neither activate nor dimerize RNase L, did not induce gene expression. Evidence for the involvement of the C-terminal region of

RNase L in dimerization was obtained by expressing truncated forms of RNase L. These findings describe a convenient, high-throughput screening method for RNase L activators which could lead to the discovery of novel anti-viral and anti-cancer agents.

L5 ANSWER 44 OF 74 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 1998105756 MEDLINE
DOCUMENT NUMBER: 98105756 PubMed ID: 9445011
TITLE: Regulation of human immunodeficiency virus replication by 2',5'-oligoadenylate-dependent RNase L.
AUTHOR: Maitra R K; Silverman R H
CORPORATE SOURCE: Virus Core Facility, The Lerner Research Institute, The Cleveland Clinic Foundation, Ohio 44195, USA.
CONTRACT NUMBER: CA 44059 (NCI)
SOURCE: JOURNAL OF VIROLOGY, (1998 Feb) 72 (2) 1146-52.
Journal code: KCV; 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980226
Last Updated on STN: 19980226
Entered Medline: 19980218

AB Activation of **RNase L** by 2',5'-linked oligoadenylates (2-5A) is one of the antiviral pathways of interferon action. To determine the involvement of the 2-5A system in the control of human immunodeficiency virus type 1 (HIV-1) replication, a segment of the HIV-1 nef gene was replaced with human **RNase L** cDNA. HIV-1 provirus containing sense orientation **RNase L** cDNA caused increased expression of **RNase L** and 500- to 1,000-fold inhibition of virus replication in Jurkat cells for a period of about 2 weeks. Subsequently, a partial deletion of the **RNase L** cDNA which coincided with increases in virus production occurred. The anti-HIV activity of **RNase L** correlated with decreases in HIV-1 RNA and with an acceleration in cell death accompanied by DNA fragmentation. Replication of HIV-1 encoding **RNase L** was also transiently suppressed in peripheral blood lymphocytes (PBL). In contrast, recombinant HIV containing reverse orientation **RNase L** cDNA caused decreased levels of **RNase L**, increases in HIV yields, and reductions in the anti-HIV effect of alpha interferon in PBL and in Jurkat cells. To obtain constitutive and continuous expression of **RNase L** cDNA, Jurkat cells were cotransfected with HIV-1 proviral DNA and with plasmid containing a cytomegalovirus promoter driving expression of **RNase L** cDNA. The **RNase L** plasmid suppressed HIV-1 replication by eightfold, while an **antisense RNase L** construct enhanced virus production by twofold. These findings demonstrate that **RNase L** can severely impair HIV replication and suggest involvement of the 2-5A system in the anti-HIV effect of alpha interferon.

L5 ANSWER 45 OF 74 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 1999081091 MEDLINE
DOCUMENT NUMBER: 99081091 PubMed ID: 9865493
TITLE: Selective mRNA degradation by **antisense** oligonucleotide-2,5A chimeras: involvement of RNase H and **RNase L**.
AUTHOR: Robbins I; Mitta G; Vichier-Guerre S; Sobol R; Ubysz A; Rayner B; Lebleu B
CORPORATE SOURCE: Institut de Genetique Moleculaire de Montpellier, CNRS, UMR 5535, Universite de Montpellier II, France.
SOURCE: BIOCHIMIE, (1998 Aug-Sep) 80 (8-9) 711-20.

Journal code: A14; 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990402
Last Updated on STN: 19990402
Entered Medline: 19990324

AB **Antisense** oligonucleotides (ON) allow the specific control of gene expression and phosphorothioate derivatives are currently being evaluated for possible clinical applications. Numerous second generation ON analogues with improved pharmacological properties have been described.

Most of them, however, do not recruit RNase H, which is known to increase ON potency by eliciting the specific degradation of the target RNA. Silverman, Torrence and colleagues have conjugated 2,5A to natural **antisense** ON and demonstrated the preferential cleavage of a target RNA in cell-free and intact cell experiments. We have established for the first time that RNase H-incompetent ON, viz. alpha-anomeric ON analogues, can be converted into sequence-specific nucleases upon conjugation to 2,5A. The use of alpha-ON- and beta-ON-2,5A chimeras has allowed us to delineate the part played by RNase H and **RNase L** in target RNA degradation and translation arrest. Finally, the present studies have revealed limitations which are encountered in the choice of a suitable target for such ON-2,5A chimeras.

L5 ANSWER 46 OF 74 MEDLINE DUPLICATE 29
ACCESSION NUMBER: 1998321614 MEDLINE
DOCUMENT NUMBER: 98321614 PubMed ID: 9660177
TITLE: **RNase L** inhibitor (RLI) **antisense** constructions block partially the down regulation of the 2-5A/**RNase L** pathway in encephalomyocarditis-virus-(EMCV)-infected cells.
AUTHOR: Martinand C; Salehzada T; Silhol M; Lebleu B; Bisbal C
CORPORATE SOURCE: Molecular Genetics Institute, UMR 5535, CNRS Montpellier, France.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1998 Jun 1) 254 (2) 248-55.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980731
Last Updated on STN: 19980731
Entered Medline: 19980721

AB The interferon-(IFN)-inducible 2',5'-oligoadenylate (2-5A)/endoribonuclease L (**RNase L**) pathway plays a major role in the antiviral and antiproliferative effects of IFN. The 2-5A/**RNase L** pathway appears to be regulated by the cell-growth status or viral infection. Viruses, and picornaviruses in particular, have evolved strategies to escape the 2-5A/**RNase L**-pathway-associated antiviral activity. We have recently cloned a cDNA coding for RLI, a **RNase-L**-specific protein inhibitor. Its regulated expression by viral infection could provide a new strategy to modulate the 2-5A/**RNase L** pathway. Since **RNase L** had been shown to be down regulated upon encephalomyocarditis (EMCV) infection, we stably transfected HeLa cells with a RLI **antisense** cDNA expressing vector. Four independent clones named VAS1, VAS2, VAS3 and VAS4 and one clone transfected with the empty vector (VV) as control, were analyzed. The level of RLI was decreased by 20% for VAS1, 25% for VAS2, 75% for VAS3 and 50% for VAS4. The inactivation of **RNase L** observed during EMCV

infection was decreased in these clones as compared to control HeLa cells.

Here again the results vary between the four clones. The maximum inhibition of **RNase L** (90%) was observed in control cells and in VAS1 while 48% inhibition was observed in VAS4 and 25% in VAS3. The reversal in **RNase L** inhibition thus reflects closely the resulting RLI level, in keeping with a major role of RLI in EMCV-induced down regulation of 2-5A-binding activity of **RNase L**. Moreover, cells expressing a low level of RLI (VAS3 and VAS 4) are partially resistant to EMCV infection.

L5 ANSWER 47 OF 74 MEDLINE DUPLICATE 30
ACCESSION NUMBER: 1998410629 MEDLINE
DOCUMENT NUMBER: 98410629 PubMed ID: 9735309
TITLE: Ribonuclease L, a 2-5A-dependent enzyme: purification to homogeneity and assays for 2-5A binding and catalytic activity.
AUTHOR: Player M R; Wondrak E M; Bayly S F; Torrence P F
CORPORATE SOURCE: Laboratory of Medicinal Chemistry, Building 8, Room B2A02, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, 20892-0805, USA.
SOURCE: METHODS, (1998 Jul) 15 (3) 243-53.
Journal code: CPO; 9426302. ISSN: 1046-2023.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981029

AB **RNase L** is a latent endonuclease found in reptiles, birds, and mammals. It is activated by the 2',5'-phosphodiester-linked oligoadenylates called 2-5A and has been implicated in the mechanism of action of interferon, as well as in a variety of other biological phenomena such as apoptosis. Covalent linkage of 2-5A to **antisense** oligonucleotides permits recruitment of **RNase L** for enhancement of **antisense** action. The purification of **RNase L** described herein and the assays for its detection and activation will help to provide further mechanistic details on how this unique nuclease functions and what its biochemical roles may be. In addition, such assays will facilitate the screening of 2-5A-**antisense** congeners for exploration of the potential therapeutic applications of **RNase L**.


L5 ANSWER 48 OF 74 MEDLINE DUPLICATE 31
ACCESSION NUMBER: 1999092560 MEDLINE
DOCUMENT NUMBER: 99092560 PubMed ID: 9875401
TITLE: Targeting **RNase L** to human immunodeficiency virus RNA with 2-5A-**antisense**.
AUTHOR: Player M R; Maitra R K; Silverman R H; Torrence P F
CORPORATE SOURCE: Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0805, USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
SOURCE: ANTIVIRAL CHEMISTRY AND CHEMOTHERAPY, (1998 May) 9 (3) 225-31.
Journal code: C79; 9009212. ISSN: 0956-3202.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216

Entered Medline: 19990202

AB In an attempt to develop a lead for the application of 2-5A-**antisense** to the targeted destruction of human immunodeficiency virus (HIV) RNA, specific target sequences within the HIV mRNAs were identified by analysis of the theoretical secondary structure. 2-5A-**antisense** chimeras were chosen against a total of 11 different sequences: three in the gag mRNA, three in the rev mRNA and five in the tat mRNA. 2-5A-**antisense** chimera synthesis was accomplished using solid-phase phosphoramidite chemistry. These chimeras were evaluated for their activity in a cell-free assay system using purified recombinant human **RNase L** to effect cleavage of 32P-labelled RNA transcripts of plasmids derived from HIV NL4-3. This screening revealed that of the three 2-5A-**antisense** chimeras targeted against gag mRNA, only one had significant HIV RNA cleavage activity, approximately 10-fold-reduced compared to the parent 2-5A tetramer and comparable to that reported for the prototypical 2-5A-anti-PKR chimera, targeted against PKR mRNA. The cleavage activity of this chimera was specific, since a scrambled **antisense** domain chimera and a chimera without the key 5'-monophosphate moiety were both inactive. The 10 other 2-5A-**antisense** chimeras against tat and rev had significantly less activity. These results imply that HIV gag RNA, like PKR RNA and a model HIV tat-oligoA-vif RNA, can be cleaved using the 2-5A-**antisense** approach. The results further imply that not all regions of a potential RNA target are accessible to the 2-5A-**antisense** approach.

L5 ANSWER 49 OF 74 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:557648 CAPLUS

DOCUMENT NUMBER: 127:215199

TITLE: Compositions containing **RNase L** activators conjugated to **antisense** oligonucleotides for treatment of respiratory syncytial virus infections

INVENTOR(S): Torrence, Paul F.; Silverman, Robert H.; Cirino, Nick M.; Li, Guiying; Xiao, Wei

PATENT ASSIGNEE(S): National Institutes of Health, USA; Cleveland Clinic Foundation

SOURCE: PCT Int. Appl., 88 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9729757 | A1 | 19970821 | WO 1997-US2531 | 19970214 |
| W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | | |
| CA 2246503 | AA | 19970821 | CA 1997-2246503 | 19970214 |
| AU 9721298 | A1 | 19970902 | AU 1997-21298 | 19970214 |
| AU 708535 | B2 | 19990805 | | |
| CN 1215994 | A | 19990505 | CN 1997-193797 | 19970214 |
| JP 2000506384 | T2 | 20000530 | JP 1997-529569 | 19970214 |
| EP 1007655 | A1 | 20000614 | EP 1997-906662 | 19970214 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |

PRIORITY APPLN. INFO.: US 1996-11725P P 19960215
WO 1997-US2531 W 19970214

AB The invention concerns compds. and methods for treating infection with

Respiratory Syncytial Virus. The compds. comprise an **antisense** portion, which is complementary to a normally single stranded portion of the RSV antigenomic strand (the mRNA strand), a linker, and an oligonucleotide activator of **RNase L**, a ubiquitous non-specific RNase. The method comprises forming a complex of an activated **RNase L** and the **antisense** mol.
The application teaches methods of detg. which portions of the RSV antigenomic strand are normally single-stranded. The application teaches that an **antisense** oligonucleotide having the sequence of residues 8281-8299 of the RSV genome is particularly useful to practice the invention and provides in vitro results superior to those obtainable with the conventional drug of choice, ribavirin.

L5 ANSWER 50 OF 74 USPATFULL

| | |
|---|---|
| ACCESSION NUMBER: | 97:94222 USPATFULL |
| TITLE: | Method of cleaving specific strands of RNA and medical treatments thereby |
| INVENTOR(S): | Torrence, Paul, Silver Spring, MD, United States<br>Silverman, Robert, Shaker Heights, OH, United States<br>Maitra, Ratan, Euclid, OH, United States<br>Lesiak, Krystyna, Gaithersburg, MD, United States |
| PATENT ASSIGNEE(S): | The Cleveland Clinic Foundation, Cleveland, OH, United States (U.S. corporation)<br>The United States of America, Washington, DC, United States (U.S. government) |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5677289 | | 19971014 |
| APPLICATION INFO.: | US 1995-458050 | | 19950601 (8) |
| RELATED APPLN. INFO.: | Continuation of Ser. No. US 1993-123449, filed on 17 Sep 1993, now patented, Pat. No. US 5583032 which is a continuation-in-part of Ser. No. US 1992-965666, filed on 21 Oct 1992, now abandoned | | |
| DOCUMENT TYPE: | Utility | | |
| FILE SEGMENT: | Granted | | |
| PRIMARY EXAMINER: | Rories, Charles C.P. | | |
| LEGAL REPRESENTATIVE: | Pennie & Edmonds LLP | | |
| NUMBER OF CLAIMS: | 11 | | |
| EXEMPLARY CLAIM: | 1 | | |
| NUMBER OF DRAWINGS: | 12 Drawing Figure(s); 12 Drawing Page(s) | | |
| LINE COUNT: | 2414 | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of using a chimeric molecule made up of an antisense oligonucleotide attached to a 2',5'-oligoadenylate molecule to specifically cleave a sense strand of RNA, wherein the antisense oligonucleotide of the chimeric molecule is hybridized to the sense strand of RNA in the presence of 2',5'-dependent RNase.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 51 OF 74 USPATFULL

| | |
|---|---|
| ACCESSION NUMBER: | 97:59104 USPATFULL |
| TITLE: | C-myb targeted ribozymes |
| INVENTOR(S): | Stinchcomb, Dan T., Boulder, CO, United States<br>Draper, Kenneth, Boulder, CO, United States<br>McSwiggen, James, Boulder, CO, United States<br>Jarvis, Thale, Boulder, CO, United States |
| PATENT ASSIGNEE(S): | Ribozyme Pharmaceuticals, Inc., Boulder, CO, United States (U.S. corporation) |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5646042 | | 19970708 |
| APPLICATION INFO.: | US 1995-373124 | | 19950113 (8) |
| RELATED APPLN. INFO.: | Continuation-in-part of Ser. No. US 1992-987132, filed | | |

on 7 Dec 1992, now abandoned And a
continuation-in-part
of Ser. No. US 1994-192943, filed on 7 Feb 1994 which is a continuation of Ser. No. US 1992-936422, filed on 26 Aug 1992, now abandoned And a continuation of Ser. No. US 1994-245466, filed on 18 May 1994, now
abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Leguyader, John L.
LEGAL REPRESENTATIVE: Lyon & Lyon
NUMBER OF CLAIMS: 220
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 29 Drawing Figure(s); 19 Drawing Page(s)
LINE COUNT: 4869
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Enzymatic nucleic acid molecules which cleave c-myb RNA or other RNAs associated with restenosis or cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 52 OF 74 USPATFULL
ACCESSION NUMBER: 97:3821 USPATFULL
TITLE: Treatment of viral hepatitis with mismatched dsRNA
INVENTOR(S): Carter, William A., Birchrunville, PA, United States
PATENT ASSIGNEE(S): Hemispherx Biopharma Inc., Philadelphia, PA, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5593973 | | 19970114 |
| APPLICATION INFO.: | US 1994-318514 | | 19941005 (8) |

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-158357, filed on 29 Nov 1993, now abandoned which is a continuation of Ser.
No. US 1992-967579, filed on 27 Oct 1992, now abandoned
which is a continuation of Ser. No. US 1991-713003, filed on 10 Jun 1991, now abandoned which is a continuation of Ser. No. US 1990-560273, filed on 30 Jul 1990, now abandoned which is a continuation of Ser.
No. US 1988-237018, filed on 26 Aug 1988, now abandoned
which is a continuation-in-part of Ser. No. US 1987-93523, filed on 4 Sep 1987, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Leguyader, John L.
LEGAL REPRESENTATIVE: Nixon & Vanderhye
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 6 Drawing Figure(s); 4 Drawing Page(s)
LINE COUNT: 1280
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Hepatitis viral infections are efficaciously treated with mismatched dsRNAs, notably rI.sub.n.r(C.sub.11-14,U).sub.n.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 53 OF 74 MEDLINE DUPLICATE 32
ACCESSION NUMBER: 97203165 MEDLINE
DOCUMENT NUMBER: 97203165 PubMed ID: 9050883
TITLE: Targeting RNA decay with 2',5' oligoadenylate-antisense in respiratory syncytial virus-infected cells.
AUTHOR: Cirino N M; Li G; Xiao W; Torrence P F; Silverman R H

CORPORATE SOURCE: Department of Cancer Biology, Research Institute, The Cleveland Clinic Foundation, OH 44195, USA.
CONTRACT NUMBER: 1 PO1 CA 62220 (NCI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Mar 4) 94 (5) 1937-42. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970422
Last Updated on STN: 19970422
Entered Medline: 19970407

AB Treatment of human cells with 2',5' oligoadenylate covalently linked to **antisense** (2-5A-**antisense**) results in the selective cleavage of targeted RNA species by **2-5A-dependent RNase L**. Here we show that 2-5A-**antisense** containing stabilizing modifications at both termini are effective in suppressing the replication of respiratory syncytial virus (RSV) in human tracheal epithelial cells. The affinity of 2-5A-**antisense** for different regions in the RSV M2 and L mRNAs was predicted from a computer-generated model of the RNA secondary structure. The most potent 2-5A-**antisense** molecule caused a highly effective, dose-dependent suppression of RSV yields when added to previously infected cells. In contrast, control oligonucleotides, including an inactive dimeric form of 2-5A linked to **antisense**, 2-5A linked to a randomized sequence of nucleotides, and **antisense** molecules lacking 2-5A, had minimal effects on virus replication. The specificity of this approach was shown by reverse transcriptase-coupled PCR analysis of RSV M2, P, and N mRNA and of cellular glyceraldehyde-3-phosphate dehydrogenase mRNA. The RSV M2 mRNA amounts were depleted after treating RSV-infected cells with 2-5A-**antisense** targeted to this mRNA, whereas the amounts of the other RNA species were unchanged. These studies demonstrate that 2',5' oligoadenylate covalently linked to **antisense** (2-5A-**antisense**) can effectively suppress RSV replication by directing the cellular **RNase L** to selectively degrade an essential viral mRNA.

L5 ANSWER 54 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:758531 CAPLUS
DOCUMENT NUMBER: 128:97336
TITLE: Inhibition of respiratory syncytial virus by double termini-protected 2-5A antisense chimeras
AUTHOR(S): Xiao, Wei; Li, Guiying; Torrence, Paul F.; Cirino, Nick M.; Silverman, Robert H.
CORPORATE SOURCE: Section on Biomedical Chemistry, NIDDK, National Institutes of Health, Bethesda, MD, 20892, USA
SOURCE: Nucleosides Nucleotides (1997), 16(7-9), 1735-1738 CODEN: NUNUD5; ISSN: 0732-8311
PUBLISHER: Marcel Dekker, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Respiratory syncytial virus (RSV) replication was reduced by greater than 90% after treatment of infected human tracheal epithelial cell line, 9HTE, with double termini-protected 2-5A antisense chimeras. The anti-RSV activity of 2-5A antisense is improved by double termini protection of the chimeras. Also, the effective 2-5A antisense can be designed based on the computer-assisted anal. of sec. structure of RSV mRNA with the single-stranded large loop region as binding site. The specific 2-5A antisense functions as a very effective anti-RSV agents and have the potential to be developed as agents for the treatment of active RSV infection in humans.

L5 ANSWER 55 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:758181 CAPLUS
DOCUMENT NUMBER: 128:61756
TITLE: The synthesis of 2-5A antisense chimeras with various non-nucleoside components
AUTHOR(S): Zhang, Weifeng; Torrence, Paul
CORPORATE SOURCE: Section of Biomedical Chemistry, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney, National Institutes of Health, Bethesda, MD, 20878, USA
SOURCE: Nucleosides Nucleotides (1997), 16(7-9), 1579-1582
CODEN: NUNUD5; ISSN: 0732-8311
PUBLISHER: Marcel Dekker, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have synthesized a series of 2-5A chimeras in which the nature of the oligoadenylate-**antisense** linkage and the length of the 2',5'-oligoadenylate were vaned. In addn., a branched linker was introduced to relocate the 2',5'-oligoadenylate with respect to the **antisense** domain. The activities of title chimeras were tested against RNA-dependent protein kinase mRNA in presence of human **RNase L** in cell free system.

L5 ANSWER 56 OF 74 MEDLINE DUPLICATE 33
ACCESSION NUMBER: 97265356 MEDLINE
DOCUMENT NUMBER: 97265356 PubMed ID: 9111293
TITLE: Correlation of selective modifications to a 2',5'-oligoadenylate-3',5'-deoxyribonucleotide **antisense** chimera with affinity for the target nucleic acid and with ability to activate **RNase L**.
AUTHOR: Xiao W; Li G; Maitra R K; Maran A; Silverman R H; Torrence P F
CORPORATE SOURCE: Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0815, USA.
CONTRACT NUMBER: 1 PO1 CA62220 (NCI)
SOURCE: JOURNAL OF MEDICINAL CHEMISTRY, (1997 Apr 11) 40 (8) 1195-200.
Journal code: J0F; 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970523
Last Updated on STN: 19980206
Entered Medline: 19970509
AB The use of an **antisense** oligonucleotide to address a specific targeted RNA sequence and subsequent localized activation of the **2-5A-dependent RNase** (**RNase L**) to effect selective RNA degradation is a new approach to the control of gene expression called 2-5A-**antisense**. The previously reported biological activity of the 2-5A:AS chimeric oligonucleotide [p5'(A2'p)3A-antiPKR1], directed against nucleotides 55-73 of the coding sequence of the PKR mRNA, has been used as a point of reference to examine the effect of introducing mismatches into the chimeric oligonucleotide, altering the chain length of the **antisense** domain of the chimeras, removal of the 5'-monophosphate moiety, shortening the 2',5'-oligoadenylate domain, and substitution of 3',5'-linked 2'-deoxyadenosine nucleotides for the 2-5A domain. The general formula for the novel chimeric oligonucleotides is p5'(A2'p)3A2'p(CH2)4p(CH2)4p(5'N3'p

)mN, where N is any nucleoside and m is any integer. When the biological activity of these new chimeric oligonucleotides was compared to that of the parent chimera, 2-5A-aPKR, for their ability to effect target PKR RNA cleavage in a cell-free and in an intact cell assay, it was determined that there was a close correlation between the activity of 2-5A-**antisense** chimeras and their affinity (Tm) for a targeted nucleic acid. In addition, there was also a close correlation between activity of the 2-5A-**antisense** chimeras and their ability to activate the **2-5A-dependent RNase L**.

L5 ANSWER 57 OF 74 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1998:61845 BIOSIS
DOCUMENT NUMBER: PREV199800061845
TITLE: Specific degradation of BCR/ABL mRNA and growth suppression of CML cells by 2-5A antisense oligonucleotides.
AUTHOR(S): Waller, Cornelius F. (1); Maran, Avudaiappan; Paranjape, Jayashree M.; Li, Guiying; Xiao, Wei; Zhang, Kerry; Kalaycioglu, Matt; Torrence, Paul F.; Silverman, Robert H.
CORPORATE SOURCE: (1) Dep. Cancer Biol., Research Inst., Cleveland Clin. Found., Cleveland, OH USA
SOURCE: Blood, (Nov. 15, 1997) Vol. 90, No. 10 SUPPL. 1 PART 2, pp. 283B.
Meeting Info.: Thirty-ninth Annual Meeting of the American Society of Hematology San Diego, California, USA December 5-9, 1997 The American Society of Hematology
. ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English

L5 ANSWER 58 OF 74 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 34
ACCESSION NUMBER: 1997:411628 CAPLUS
DOCUMENT NUMBER: 127:131622
TITLE: Recruiting the 2-5A system for antisense therapeutics
AUTHOR(S): Torrence, Paul F.; Xiao, Wei; Li, Guiying; Cramer, Hagen; Player, Mark R.; Silverman, Robert H.
CORPORATE SOURCE: Section Biomedical Chem., Lab. Medicinal Chem., National Inst. Diabetes Digestive Kidney Diseases, National Inst. Health, Bethesda, MD, 20892-0805, USA
SOURCE: Antisense Nucleic Acid Drug Dev. (1997), 7(3), 203-206
CODEN: ANADF5; ISSN: 1087-2906
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have explored a targeted mRNA destruction method that derives from the covalent linkage of a 3',5'-**antisense** oligodeoxyribonucleotide and a 2',5'-oligoadenylate activator of **RNase L**, the **2-5A-dependent RNase** (Torrence et at., 1993; Lesiak et al., 1993), a novel RNase assocd. with interferon (IFN) action (Johnston and Totrence, 1984). This composite nucleic acid could, through the **antisense** domain, target the chimera to a particular mRNA sequence, which would then be targeted for destruction by the 2-5A component, which would provide a localized activation of the latent **2-5A-dependent RNase.** The 2-5A-**antisense** approach to specific nucleic acid cleavage has a no. of significant advantages when compared with other approaches to targeted cellular RNA degrdn. First, it relies on a nuclease activity that is endogenous and ubiquitous in mammalian cells but is active only when bound to 2-5A. Second, the substrate specificity of **RNase L** appears susceptible to modulation through changes in the **antisense** cassette of the 2-5A-**antisense** chimera. Third, in contrast to a no. of other strategies, the DNA:RNA hybrid formed presumably would still be susceptible to attack by RNase H also. However,

DNA chain modifications, such as methylphosphonate introduction, although eliminating RNase H-catalyzed scission as a mode of degrdn., would not be expected to affect **2-5A-dependent**
**RNase** activation ability. The recruitment of an entirely new and different nuclease for the targeted destruction of RNA should greatly expand the range and potential of **antisense** therapeutics.

L5 ANSWER 59 OF 74 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 1997:239206 BIOSIS
DOCUMENT NUMBER: PREV199799538409
TITLE: Anti-respiratory syncytial virus (RSV) activity of 2-5a antisense oligonucleotide chimeras.
AUTHOR(S): Barnard, D. L. (1); Sidwell, R. W. (1); Matheson, J. E. (1); Xiao, W.; Player, M.; Torrence, P. F.
CORPORATE SOURCE: (1) Inst. Antiviral Res., Utah State Univ., Logan, UT USA
SOURCE: Antiviral Research, (1997) Vol. 34, No. 2, pp. A89.
Meeting Info.: Meeting of the International Society for Antiviral Research and the Tenth International Conference on Antiviral Research Atlanta, Georgia, USA April 6-11, 1997
ISSN: 0166-3542.
DOCUMENT TYPE: Conference; Abstract; Conference
LANGUAGE: English

L5 ANSWER 60 OF 74 MEDLINE
ACCESSION NUMBER: 96389590 MEDLINE
DOCUMENT NUMBER: 96389590 PubMed ID: 8796884
TITLE: **RNase L** and 2-5A to enhance **antisense** technology and target the destruction of mRNA.
AUTHOR: Glaser V
SOURCE: MOLECULAR MEDICINE TODAY, (1996 May) 2 (5) 183.
Journal code: CMK; 9508560. ISSN: 1357-4310.
PUB. COUNTRY: ENGLAND: United Kingdom
News Announcement
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 19961025
Entered Medline: 19961016

L5 ANSWER 61 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:130011 CAPLUS
DOCUMENT NUMBER: 126:129382
TITLE: Virus-resistant transgenic plants with a functional human 2'.fwdarw.5' oligoadenylic acid polymerase and RNase L
INVENTOR(S): Silverman, Robert H.; Mitra, Amitava
PATENT ASSIGNEE(S): Cleveland Clinic Foundation, USA
SOURCE: PCT Int. Appl., 187 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9639806 | A1 | 19961219 | WO 1996-US9895 | 19960607 |

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML

| | | | | | |
|---|---|---|---|---|---|
| US 5866787 | A | 19990202 | US 1995-487797 | 19950607 |
| AU 9663827 | A1 | 19961230 | AU 1996-63827 | 19960607 |
| EP 836377 | A1 | 19980422 | EP 1996-923267 | 19960607 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| JP 11507232 | T2 | 19990629 | JP 1996-502075 | 19960607 |
| BR 9608588 | A | 19990914 | BR 1996-8588 | 19960607 |

PRIORITY APPLN. INFO.:

| | |
|---|---|
| US 1995-487797 | 19950607 |
| US 1993-28086 | 19930308 |
| US 1994-198973 | 19940218 |
| WO 1996-US9895 | 19960607 |

AB Novel transgenic plants expressing the human genes for a (2'.fwdarw.5')-Oligo(A) synthetase, that produces 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) in response to double-stranded RNA (dsRNA), and a 2-5A-dependent (RNase L), are disclosed. These plants, e.g. tobacco, are resistant to viral infection. When transgenic tobacco plants expressing these genes are exposed to three different types of plant viruses, i.e., tobacco mosaic virus, tobacco etch virus and alfalfa mosaic virus, such viral exposure leads to necrotic local lesions in such transgenic tobacco plants instead of typical systemic infections.

L5 ANSWER 62 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:388341 CAPLUS
DOCUMENT NUMBER: 125:52388
TITLE: RNase L inhibitor and nucleic acid encoding it and preparation of anti-viral agents
INVENTOR(S): Salehzada, Tamim; Bisbal, Catherine
PATENT ASSIGNEE(S): Centre National de la Recherche Scientifique, Fr.; Institut National de la Sante et de la Recherche Medicale (INSERM)
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9610636 | A1 | 19960411 | WO 1995-FR1277 | 19951002 |
| W: CA, JP, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| FR 2725214 | A1 | 19960405 | FR 1994-11752 | 19940930 |

PRIORITY APPLN. INFO.:

| | |
|---|---|
| FR 1994-11752 | 19940930 |

AB Nucleotide sequences capable of coding for polypeptides having RNase L inhibitory activity (RLI) are disclosed. Said nucleotide sequences and said inhibitors are useful for developing antiviral agents. CDNA for human RLI was cloned and sequenced. RLI gene expression was induced by some viruses, e.g. encephalomyocarditis virus, HIV. Interferons induced RNase L prodn., but not RLI prodn. RLI functions by binding to RNase L and preventing 2-5A binding, not by degrading 2-5A itself. The RLI gene was localized to chromosome 4q31. The protein sequence contained the motif CX2CS2CX3C found in ferredoxin.

L5 ANSWER 63 OF 74 USPATFULL
ACCESSION NUMBER: 96:113828 USPATFULL
TITLE: Method of cleaving specific strands of RNA
INVENTOR(S): Torrence, Paul, Silver Spring, MD, United States
Silverman, Robert, Shaker Heights, OH, United States
Maitra, Ratan, Euclid, OH, United States
Lesiak, Krystyna, Gaithersburg, MD, United States
PATENT ASSIGNEE(S): The Cleveland Clinic Foundation and National Institutes
of Health, Bethesda, MD, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 5583032 | | 19961210 |
| APPLICATION INFO.: | US 1993-123449 | | 19930917 (8) |

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1992-965666, filed on 21 Oct 1992, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Rories, Charles C. P.
LEGAL REPRESENTATIVE: Pennie & Edmonds
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 2560
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method of using a chimeric molecule made up of an antisense oligonucleotide attached to a 2',5'-oligoadenylate molecule to specifically cleave a sense strand of RNA, wherein the antisense oligonucleotide of the chimeric molecule is hybridized to the sense strand of RNA in the presence of 2',5'-dependent RNase.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 64 OF 74 MEDLINE DUPLICATE 35
ACCESSION NUMBER: 97165098 MEDLINE
DOCUMENT NUMBER: 97165098 PubMed ID: 9012860
TITLE: Synthesis and characterization of composite nucleic acids containing 2', 5'-oligoriboadenylate linked to antisense DNA.
AUTHOR: Xiao W; Player M R; Li G; Zhang W; Lesiak K; Torrence P F
CORPORATE SOURCE: Section on Biomedical Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0805, USA.
SOURCE: ANTISENSE AND NUCLEIC ACID DRUG DEVELOPMENT, (1996 Winter) 6 (4) 247-58.
Journal code: CJY; 9606142. ISSN: 1087-2906.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970424
Last Updated on STN: 19980206
Entered Medline: 19970415

AB Composite nucleic acids, known as 2-5A **antisense** chimeras, cause the 2-5A-dependent ribonuclease (**RNase L**) to catalyze the specific cleavage of RNA in cell free systems and in intact cells. Such 2-5A **antisense** chimeras are 5'-monophosphorylated, 2,'5'-linked oligoadenylates covalently attached to **antisense** 3',5'-oligodeoxyribonucleotides by means of a linker containing two residues of 1,4-butanediol phosphate. Here we report a fully automated synthesis of 2-5A **antisense** chimeras on a solid support using phosphoramidite methodology with specific coupling time modifications and their subsequent purification by reverse-phase ion-pair and anion exchange HPLC. Purified 2-5A **antisense** chimeras were characterized by [1H]NMR and [31P]NMR, MALDIMS, and capillary gel electrophoresis. The synthetic 2',5'-linked oligoadenylate showed no phosphodiester isomerization to 3',5' during or after synthesis. In addition, we have developed facile methodologies to characterize the chimeras using digestion with various hydrolytic enzymes including snake venom phosphodiesterase I and nuclease P1. Finally, Maxam-Gilbert chemical sequencing protocols have been developed to confirm the entire sequence of these chimeric oligonucleotides.

L5 ANSWER 65 OF 74 MEDLINE DUPLICATE 36
ACCESSION NUMBER: 95318066 MEDLINE
DOCUMENT NUMBER: 95318066 PubMed ID: 7797490
TITLE: Catalytic cleavage of an RNA target by 2-5A **antisense** and **RNase L**.
AUTHOR: Maitra R K; Li G; Xiao W; Dong B; Torrence P F; Silverman R H
CORPORATE SOURCE: Department of Cancer Biology, Cleveland Clinic Foundation, Ohio 44195, USA.
CONTRACT NUMBER: 1 PO1 CA 62220-01A1 (NCI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jun 23) 270 (25) 15071-5.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950817
Last Updated on STN: 19980206
Entered Medline: 19950731

AB 2-5A **antisense** (2-5A-AS) molecules are chimeric oligonucleotides that cause **2-5A-dependent RNase** (**RNase L**) to catalyze the selective cleavage of RNA in human cells. These composite nucleic acids consist of a 5'-monophosphorylated, 2',5'-linked oligoadenylate known as 2-5A (an activator of **RNase L**) covalently attached to **antisense** 3',5'-oligodeoxyribonucleotides. Here, we characterize the targeted cleavage of the double-stranded RNA-dependent protein kinase (PKR) mRNA by purified, recombinant human **RNase L**. A 2-5A-AS chimera, which contains complementary sequence to PKR mRNA, and unmodified 2-5A, which causes general RNA decay, were about 20- and 40-fold more active, respectively, than 2-5A-AS chimeras in which the DNA domains are not complementary to sequences in PKR mRNA. Directed cleavage was efficient because each 2-5A-AS chimera targeted many RNA molecules. Moreover, **RNase L** caused the catalytic cleavage of the RNA target (kcat of approximately 7 s-1). The precise sites of PKR mRNA cleavage caused by 2-5A-AS were mapped, using a primer extension assay, to phosphodiester bonds adjacent to the 3' terminus of the chimera binding site (5' on the RNA target) as well as within the chimera's oligonucleotide binding site itself. The selectivity of this approach is shown to be provided by the **antisense** arm of the chimera, which places the RNA target in close proximity to the RNase.

L5 ANSWER 66 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:74025 CAPLUS
DOCUMENT NUMBER: 124:193016
TITLE: 2-5A-antisense: A novel approach to cancer therapy
AUTHOR(S): Waller, Cornelius F.; Maitra, Ratan K.; Maran, Avudaiappan; Kumar, Aseem; Dong, Beihua; Xiao, Wei; Li, Guiying; Williams, Bryan R. G.; Torrence, Paul F.; Silverman, Robert H.
CORPORATE SOURCE: Department Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH, USA
SOURCE: Biol. Renal Cell Carcinoma, [Proc. Symp.], 3rd (1995), Meeting Date 1994, 133-48. Editor(s): Bukowski, Ronald M.; Finke, James H.; Klein, Eric A. Springer: New York, N. Y.
CODEN: 62GUAA
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English

AB A review, with 60 refs. To improve the efficiency and potency of

**antisense** RNA, a novel type of **antisense** RNA chimera with **2-5A-dependent RNase** was prepd. to degrade mRNA targets. This is a novel approach of mRNA's for disease-causing proteins. The **antisense** part of the 2-5A-**antisense** chimeras converts a nonspecific RNase into a highly specific RNase capable of cleaving individual mRNA targets in cells. In addn., the 2-5A-**antisense** chimeras provide an addnl. mechanism of **antisense** action and greatly increase the efficiency and potency of **antisense** RNA. The advantages of this technol. are the versatility, selectivity, and efficiency with which RNA targets are cleaved. Target mRNA's in human cancers will be a focus for future efforts aimed at adapting this technol. to human diseases.

L5 ANSWER 67 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:527047 CAPLUS
DOCUMENT NUMBER: 121:127047
TITLE: Method of cleaving specific strands of RNA with chimeric 2',5'-oligoadenylate-antisense oligonucleotide conjugate and pharmaceutical compositions containing the chimeras
INVENTOR(S): Torrence, Paul; Silverman, Robert; Maitra, Ratan; Lesiak, Krystyna
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;
Cleveland Clinic Research Institute
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 9409129 | A2 | 19940428 | WO 1993-US10103 | 19931020 |
| WO 9409129 | A3 | 19940526 | | |
| W: AU, CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| US 965666 | A0 | 19930401 | US 1992-965666 | 19921021 |
| US 5583032 | A | 19961210 | US 1993-123449 | 19930917 |
| AU 9455858 | A1 | 19940509 | AU 1994-55858 | 19931020 |
| AU 669250 | B2 | 19960530 | | |
| EP 666910 | A1 | 19950816 | EP 1994-901178 | 19931020 |
| EP 666910 | B1 | 20020130 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 08502408 | T2 | 19960319 | JP 1993-510391 | 19931020 |

PRIORITY APPLN. INFO.: US 1992-965666 A 19921021
US 1993-123449 A 19930917
WO 1993-US10103 W 19931020

AB A method of using a chimeric mol. made up of an antisense oligonucleotide attached to a 2',5'-oligoadenylate mol. to specifically cleave a sense strand of RNA, wherein the antisense oligonucleotide of the chimeric mol. is hybridized to the sense strand of RNA in the presence of 2',5'-dependent RNase is described. The described chimera may be used to treat various diseases (no data), e.g., cancer, or those caused by viral infection. The method was demonstrated in vivo using 2-5A linked to an 18-mer antisense oligonucleotide targetted to PKR protein mRNA. Addn. of this chimeric mol. to HeLa cells specifically destroyed PKR protein mRNA. The cells did not have to be treated in any special way in order to get the chimeras into the cells. 5'-Thiophosphorylation or addn. of an alkylamine moiety to the 3' hydroxyl of the antisense oligonucleotide provided analogs of the chimeric mol. which were equally active.

L5 ANSWER 68 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1994:549036 CAPLUS

DOCUMENT NUMBER: 121:149036
TITLE: Blockage of NF-.kappa.B signaling by selective ablation of an mRNA target by 2-5A antisense chimeras
AUTHOR(S): Maran, Avudaiappan; Maitra, Ratan K.; Kumar, Aseem; Dong, Beihua; Xiao, Wei; Li, Guiying; Williams, Bryan R. G.; Torrence, Paul F.; Silverman, Robert H.
CORPORATE SOURCE: Dep. Cancer Biol., Cleveland Clinic Foundation, Cleveland, OH, 44192, USA
SOURCE: Science (Washington, D. C.) (1994), 265(5173), 789-92
CODEN: SCIEAS; ISSN: 0036-8075
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Activation of **2-5A-dependent RNase** by 5'-phosphorylated, 2',5'-linked oligoadenylates, known as 2-5A, is one pathway of interferon action. Unaided uptake into HeLa cells of 2-5A linked to an **antisense** oligonucleotide resulted in the selective ablation of mRNA for the double-stranded RNA (dsRNA)-dependent protein kinase PKR. Similarly, purified, recombinant human **2-5A -dependent RNase** was induced to selectively cleave PKR mRNA. Cells depleted of PKR activity were unresponsive to activation of nuclear factor-.kappa.B (NF-.kappa.B) by the dsRNA poly(I):poly(C), which provides direct evidence that PKR is a transducer for the dsRNA signaling of NF-.kappa.B.

L5 ANSWER 69 OF 74 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 37
ACCESSION NUMBER: 1995:277718 CAPLUS
DOCUMENT NUMBER: 122:71145
TITLE: Development of 2',5'-oligonucleotides as potential therapeutic agents
AUTHOR(S): Torrence, P. F.; Xiao, W.; Li, G.; Khamnei, S.
CORPORATE SOURCE: Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 20892, USA
SOURCE: Curr. Med. Chem. (1994), 1(3), 176-91
CODEN: CMCHE7; ISSN: 0929-8673
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 142 refs. The unique 2',5'-phosphodiester bond-linked oligonucleotide known as 2-5A (pn5'A2'(p5'A2')mp5'A) plays a key role in mediation of the anti-encephalomyocarditis virus action of interferon. 2-5A acts as a potent inhibitor of translation through the activation of a constituent latent endonuclease, the **2-5A-dependent RNase**, which degrades RNAs. This 2-5A system, as part of a natural defense mechanism against virus infection, provides a paradigm for a new approach to the regulation of gene expression. Realization of this potential requires an understanding of the 2-5A oligoribonucleotide-assocd. structural parameters which govern its lifetime in biol. systems and its interaction with the **2-5A-dependent RNase** responsible for RNA destruction. In this review, we describe the partial realization of such an understanding and the resulting development of a new approach to the specific and targeted cleavage of RNA by directing **2-5A -dependent RNase** action to a precise target with an **antisense** DNA. The synthesis and mechanism of action of these novel composite nucleic acids permits exploration of the potent RNA destruction ability of the **2-5A-dependent RNase** coupled with the specificity of **antisense** oligonucleotides as potential therapeutic agents for a variety of diseases.

L5 ANSWER 70 OF 74 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1995:274079 CAPLUS
DOCUMENT NUMBER: 122:258690
TITLE: 2',5'-Oligoadenylate antisense chimeras for targeted

ablation of RNA

AUTHOR(S): Torrence, Paul F.; Xiao, Wei; Li, Guiying; Lesiak, Krystyna; Khamnei, Shahrzad; Maran, Avudaiappan; Maitra, Ratan; Dong, Beihua; Silverman, Robert H.

CORPORATE SOURCE: Natl. Inst. Diabetes Dig. Kidney Dis., Natl. Inst. Health, Bethesda, MD, 20892, USA

SOURCE: ACS Symp. Ser. (1994), 580(Carbohydrate Modifications in Antisense Research), 118-32
CODEN: ACSMC8; ISSN: 0097-6156

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 96 refs. The unique 2',5'-phosphodiester bond-linked oligonucleotide known as 2-5A (pn5'A2'(p5'A2')mp5'A) plays a key role in mediation of the anti-encephalomyocarditis virus action of interferon. 2-5A acts as a potent inhibitor of translation through the activation of a constituent latent endonuclease, the **2-5A-dependent RNase**, which degrades RNAs. Covalent linkage of the tetrameric p5'A2'p5'A2'p5'A to an **antisense** deoxyribonucleotide provided an adduct which was unimpaired in activation of the **2-5A-dependent RNase** and which annealed to the complementary sense DNA to give a hybrid complex with a melting temp. similar to the unmodified DNA **antisense**/sense duplex. Such 2-5A-**antisense** chimeras targeted to a modified HIV mRNA or to the dsRNA-dependent protein kinase (PKR) mRNA induced specific cleavage in their targets without affecting non-targeted mRNA species. The unaided uptake of 2-5A-**antisense** against the PKR mRNA in HeLa cells resulted in ablation of the PKR mRNA, with no effect on .beta.-actin mRNA. These findings demonstrate that 2-5A-**antisense** chimeras are effective and versatile reagents for the catalytic destruction of targeted RNA.

L5 ANSWER 71 OF 74 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:575370 CAPLUS

DOCUMENT NUMBER: 119:175370

TITLE: Method of cleaving specific sequences of RNA

INVENTOR(S): Torrence, Paul F.; Silverman, Robert; Maitra, Ratan K.; Lesiak, Krystyna

PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA

SOURCE: U. S. Pat. Appl., 29 pp. Avail. NTIS Order No. PAT-APPL-7-965,666.
CODEN: XAXXAV

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 965666 | A0 | 19930401 | US 1992-965666 | 19921021 |
| US 5583032 | A | 19961210 | US 1993-123449 | 19930917 |
| CA 2147282 | AA | 19940428 | CA 1993-2147282 | 19931020 |
| WO 9409129 | A2 | 19940428 | WO 1993-US10103 | 19931020 |
| WO 9409129 | A3 | 19940526 | | |
| W: AU, CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| AU 9455858 | A1 | 19940509 | AU 1994-55858 | 19931020 |
| AU 669250 | B2 | 19960530 | | |
| EP 666910 | A1 | 19950816 | EP 1994-901178 | 19931020 |
| EP 666910 | B1 | 20020130 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 08502408 | T2 | 19960319 | JP 1993-510391 | 19931020 |
| US 5677289 | A | 19971014 | US 1995-458050 | 19950601 |
| US 6271369 | B1 | 20010807 | US 1997-950196 | 19971014 |

PRIORITY APPLN. INFO.: US 1992-965666 B2 19921021

US 1993-123449 A 19930917
WO 1993-US10103 W 19931020
US 1995-458050 A3 19950601

AB A method for cleaving a specific sequence of RNA using a chimeric mol. comprised of **antisense** oligonucleotides and activators for **RNase L**, e.g. a 2',5'-oligonucleotide, is described. The site of cleavage can be directed by the **antisense** oligonucleotides. An oligo-dT 18-mer as an **antisense** component linked with the tetrameric 2',5'-phosphodiester-linked oligoadenylate p5'A2'p5'A2'p5'A2'p5'A was prepd. to demonstrate the site-specific interaction of the tetramer with **RNase L**. The tetramer-dependent cleavage of the RNA transcribed from the vif cDNA of human immunodeficiency virus-1 (HIV-1) with the RNase of the Daudi cell ext. was also demonstrated. The method can be used for medicament, e.g., to treat diseases assocd. with the prodn. of a viral protein.

L5 ANSWER 72 OF 74 MEDLINE DUPLICATE 38
ACCESSION NUMBER: 93165685 MEDLINE
DOCUMENT NUMBER: 93165685 PubMed ID: 7679499
TITLE: Targeting RNA for degradation with a (2'-5')oligoadenylate-antisense chimera.
AUTHOR: Torrence P F; Maitra R K; Lesiak K; Khamnei S; Zhou A; Silverman R H
CORPORATE SOURCE: Section on Biomedical Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892.
CONTRACT NUMBER: 5 R01 AI28253 (NIAID)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Feb 15) 90 (4) 1300-4. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19970203
Entered Medline: 19930316

AB **Antisense** oligonucleotides hold considerable promise both as research tools for inhibiting gene expression and as agents for the treatment of a myriad of human diseases. However, targeted destruction of RNA has been difficult to achieve in a versatile, efficient, and reliable manner. We have developed an effective strategy for cleaving unique RNA sequences with **2-5A-dependent RNase**, an endoribonuclease that mediates inhibitory effects of interferon on virus infection and is activated by 5'-phosphorylated 2'-5'-linked oligoadenylates known as 2-5A [pn5' A2'(p5' A2')mp5'A], resulting in the cleavage of single-stranded RNA predominantly after UpUp and UpAp sequences. To direct **2-5A-dependent RNase** to cleave unique RNA sequences, p5' A2' p5' A2'p5'A was covalently linked to an **antisense** oligonucleotide to yield a chimeric molecule (2-5A:AS). The **antisense** oligonucleotide component of 2-5A:AS bound a specific RNA sequence while the accompanying 2-5A component activated **2-5A-dependent RNase**, thereby causing the cleavage of the RNA in the targeted sequence. This strategy was demonstrated by inducing specific cleavage within a modified human immunodeficiency virus type 1 vif mRNA in a cell-free system from human lymphoblastoid cells. Because **2-5A-dependent RNase** is present in most mammalian cells, the control of gene expression based on this technology--including therapies for cancer, viral infections, and certain genetic diseases--can be envisioned.

L5 ANSWER 73 OF 74 MEDLINE DUPLICATE 39
ACCESSION NUMBER: 94137806 MEDLINE

DOCUMENT NUMBER: 94137806 PubMed ID: 8305516
TITLE: 2',5'-Oligoadenylate:antisense chimeras--synthesis and properties.
AUTHOR: Lesiak K; Khamnei S; Torrence P F
CORPORATE SOURCE: Section on Biomedical Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892.
SOURCE: BIOCONJUGATE CHEMISTRY, (1993 Nov-Dec) 4 (6) 467-72. Journal code: A1T; 9010319. ISSN: 1043-1802.
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AB We have synthesized a novel bioconjugate which joins an **antisense** oligonucleotide to a unique and potent inhibitor of translation,pn5'A2'(p5'A2')mp5'A(2-5A). Two residues of 4-hydroxybutyl phosphate were employed as linkers to attach the 2',5'-oligoadenylate moiety through its 2'-terminus to the 5'-terminus of the chosen **antisense** sequence, (dT)20. The syntheses were carried on a solid support according to the phosphite triester method of DNA synthesis (Letsinger, R.L., Lunsford, W.B. (1976) J. Am. Chem. Soc. 98, 3655-3661; Beaucage, S.L., and Caruthers, M.H. (1981) Tetrahedron Lett. 22, 1859-1862). The generated 2-5A **antisense** chimeras retained both the ability of the 2-5A molecule to activate the **2-5A-dependent RNase** as well as the ability of the oligo(dT) moiety to hybridize to the complementary poly(A). Moreover, the chimera, when annealed to its target nucleic acid sequence, was still effectively bound to the 2-5A-dependent nuclease. The methodology described represents a new approach to the selective modulation of mRNA expression.

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